Observations on the Morphology and Histochemistry of the Oviducts, Uterus and Placenta of the Sheep

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Thesis for the Degree of Doctor of Philosophy

by

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C O N T E N T S

INTRODUCTION

The morphology and cyclic changes in the reproductive tract of sheep have been studied by many investigators. Marshall (1903) studied the oestrous cycle and cyclic changes in the uterus; Casida and McKenzie (1931-33) the oviducts, uterus and vagina; Grant (1934) the cervix and vagina; Cole and Miller (1935) the uterus and vagina; McKenzie and Terril (1937) the oviducts, uterus and vagina; Hadek (1954-55) the oviduct and uterus; Schilling (1962) the oviducts.

The structure and cyclic changes in the oviducts received comparatively little attention. However, Casida and McKenzie (1931-33) and McKenzie and Terril (1937) report that the tubal epithelium reaches its maximum height in late oestrous and throughout metoestrous. They observe also the swollen cells of the connective tissue stroma and vacuolation during much of the dioestrous and the cytoplasmic projections from the tubal epithelium in dioestrous. Hadek (1955) describes the changes in the tubal epithelium in agreement with McKenzie *et al.* (1931 and 1937); he adds that rod-like cells appear when the cytoplasmic projections become detached.

Schilling (1962) states that the mucosal folds differ in the sheep and ox, i.e. longitudinal folds being more marked in the latter and the transverse in the former. The infundibulum is always large enough to cover the ovulation surface of the ovary. It is noticed that in animals during oestrous, the mucosal folds are markedly

hyperaemic. The muscularis at the upper ampulla consists of spiral fibres, an internal longitudinal muscle appears in the ampulla near the isthmus, and an outer longitudinal muscle of uterine origin is found only at the region of the isthmus.

The uterus of the sheep has received more attention than the oviducts and has been studied in detail in both non-pregnant and pregnant animals. For the general morphology the reader is referred to Asdell (1946) and Marshall (1956 - Vol. I). The uterine changes during the oestrous cycle are divided by Marshall (1903) into: (1) a period of rest, (2) a period of growth, (3) a period of breaking down of some of the vessels and extravasation of blood in the stroma and bleeding into the uterine cavity and, finally, (4) a period of recuperation and pigment formation, which he claims to be a result of extravasated blood in the stroma.

Cassida and McKenzie (1931-33) describe active proliferation of uterine epithelium during metoestrum. An intense leucocytic invasion of the epithelium is observed during metoestrum and early dioestrum. Vacuolisation of the proximal ends and crowding of nuclei to the distal ends of the cells of the supracotyledonary epithelium appear in early metoestrum. McKenzie and Terril (1937) mention that oedema of stroma and congestion of vessels in the uterus are greatest in the oestrous and postoestrous periods; growth and coiling of glands occur during postoestrous and early luteal stage reaching maximum activity at the mid-

luteal stage, followed by regression of the basal portions of the glands in pro-oestrous and early oestrous stages; folding of the epithelial surface is marked in the mid-luteal stage and leucocytes appear in greater numbers at the beginning of the regression of the corpus luteum.

Grant (1933) on the pigmentation of the uterine mucosa of the ewe states that it is due to the presence of true melanoblasts and not to pigment of haematogenous origin as was believed hitherto. He noticed that the melanoblasts appear in the uterus during foetal development and bear no relationship to the reproductive cycle.

Cole and Miller (1935) state that the uterine glands reach their maximum development in metoestrum. Hadek (1954-55) noticed that during metoestrum the epithelium becomes pseudostratified 32-37 mu. high and the mucosa oedematous and folded; in early dioestrous lymphocytes are found in the epithelium and in late dioestrous the epithelium becomes low 17-20 mu. Hadek (1955) describes the round pigment cells in the uterus of the ewe which have been found to contain, in addition to melanin granules, abnutzungspigmente (pigments of wear and tear) and he claims that on account of their ability to absorb and store material from neighbouring tissue the cells are regarded as phagocytes. He discusses and suggests that the small round pigment cells are of connective tissue origin and accepts that the melanin in the round cells is only acquired and not produced by the cells, giving as a reason the fact that the neighbouring tissue is rich in melano-cytes.

The reproductive tract of the sheep during pregnancy has been studied with special reference to placentation. Accordingly, the oviducts received little or no attention during gestation. Assheton's (1906) findings on the morphology of the ungulate placenta, though contradicted by many investigators, gained ground throughout the first half of the present Century. He states that - "On contact of the chorionic epithelium with the uterine epithelium a destruction of the latter takes place and no recovery is made over certain areas till after parturition and a layer of foetal tissue (trophoblast) becomes applied to the maternal tissues in the cotyledonary areas which has hitherto been believed to be uterine epithelium."

Jenkinson (1906) describes the formation of extra-cotyledons in the cow and supports previous workers that in the placenta of cow and sheep the crypts are lined by a secretory epithelium which arises by modification of the cells which clothe the surface of the non-pregnant uterus. As his work was independent of Assheton's, he wrote a postscript and criticised Assheton's findings. He disproved Assheton's statement by pointing out that the degeneration of uterine epithelium is perhaps illusory (careful preservation is necessary) and that the similarity in staining of foetal and maternal cells is not a safe criterion; on the other hand, the presence of fat and fatty debris in the lumen would certainly indicate that the cells are secretory like the uterine epithelial cells.

The Lining of the Maternal Crypts. It is generally agreed that during pregnancy the maternal crypts and inter-crypt columns are covered by an irregular layer of cells syncytial in character. The origin and nature of the lining cells remain controversial according to the findings of many investigators of different ruminants' placentae. William Wimsatt (1949) believes that the binucleate cells characteristic of ruminants' placentae are trophoblastic but denies that they produce the syncytial lining of the maternal crypts. However, Wimsatt (1950), making new histological observations on the placenta of sheep, states that the sheep differs from other ruminants in that the development of the placenta is attended by far greater destruction of maternal tissues than occurs in any other member of the group. He considers that the periodic acid FSA procedure is particularly well suited to demonstrate the genesis of the syncytium because it stains brilliantly a substance in the giant cells which serves as a marker whereby their transformations can be followed. He concludes that the giant cells leave the chorion, become attached to the endometrium and transform to the syncytium in question.

Amoroso (1951) in agreement with Assheton (1906) believes that the destruction of the uterine epithelium is brought about at first by the agency of darkly staining binucleate cells of the trophoblast which migrate through the uterine epithelium and come to lie on the underlying stroma. Amoroso (1952) admits that much uncertainty still remains regarding the nature of the lining cells. He

agrees that the observations recorded raise anew the possibility that these cells, in some species at least, are epithelial and of trophoblastic origin.

The electron microscope in the last ten years opened a new field of investigation regarding the fine structure and function of the placental tissues. Bjorkman and Bloom (1957) on the fine structure of the foetal-maternal junction in the bovine placentome describe the presence of microvilli at the contact surface between the cryptal and chorionic cells where an interdigitation is constituted. They state that - "The comparison of the conditions in the placental crypts with those in the intercotyledonary area and with those in the swine placenta indicates that the cryptal epithelium of the bovine placentome is of uterine origin as has been hinted by Wimsatt (1951)."

Hamilton, Harrison and Young (1960), finding similar results on the fine structure of the trophoblast and crypt lining in Dama, state that - "..... For these, admittedly mainly morphological, reasons we consider the limited regions of syncytial lining to be derived from the maternal cuboidal cells. If, on the other hand, they are derived from the binucleate cells it means that the latter, after invasion of the crypt lining, lose certain characteristic cytological features and take on those of the cuboidal cells of the lining proper. Neither Bjorkman and Bloom (1957) nor we have found any evidence for such a transformation."

Amoroso (1961) quotes - "The observations recorded by Bjorkman and Bloom (1957) and Hamilton *et al.* (1960),using

the electron microscope make it clear, however, that in the cow and fallow deer at least, the epithelial lining cells, the majority of which are cuboidal, are of maternal origin and not of foetal origin as was supposed. The remaining cryptal cells, the binucleates, are believed by Hamilton et al. (1960) to have migrated there from the trophoblast, whereas Bjorkman (1954) is convinced that the binucleate formation is a property of both maternal and foetal tissues. It is apparent that in the cow and fallow deer that the placental membrane according to Grosser's terminology is epithelio-chorial. Whether the sheep's placenta, which has long been considered as syndesmochorial, will continue to be so regarded, after examination with the electron microscope remains an open question."

In the intercotyledonary areas the uterine epithelium is believed to be eroded as a result of the contact of the trophoblast with the uterine mucosa. It appears to be restored about the tenth week of pregnancy (Assheton,1906) and about midway through the fourth month of pregnancy (Amoroso, 1956). The persistence of the uterine epithelium over the openings of the uterine glands and the formation of chorionic areolae opposite these openings are in agreement with Assheton (1906), Wimsatt (1950), and Amoroso (1956). They also agree that the uterine glands increase in length and complexity and appear to be functional throughout gestation. Kellas (1961) describes intraepithelial granular cells in the uterine epithelium of some ruminants during pregnancy. The feature of these cells is the occurrence of spherical intra-cytoplasmic inclusions which vary considerably in size

and number, stain with eosin and are metachromatic. He presents evidence which favours an origin of these cells from small lymphocytes.

The formation and growth of the foetal villi is described by Assheton (1906) who observed the appearance of crests of chorionic folds, which become little buds and penetrate deeply into the trophospongial tissue and give rise to the villi. He observes that the penetration is possibly due to an ingrowth of the binucleate cells which by becoming firmly attached to certain small areas of the maternal stroma retard the increase of the rapidly swelling up trophospongia. These small areas become the fundi of the crypts. Wimsatt (1950) observes that the earliest stage in the formation of the chorionic villi is marked by the appearance of a series of parallel ridges opposite each uterine caruncle. Separate prominences appear on the summits of these ridges and, by continued growth and incorporation with the intervening segments of the ridges, give rise to the primary villi. Then by terminal budding each primary villus sprouts two or more branches which, in turn, give rise to branches of the third order. Amoroso (1956) quotes - "Villi are developed as buds of the foetal ectoderm which afterwards contain cores of mesoderm with branches of the allantoic vessels. They fit into depressions or crypts on the surface of the maternal cotyledons, increase in length and branch in different directions as pregnancy advances. Whether they literally grow into the maternal tissues either mechanically or by a phagocytic action is uncertain. It seems that the sub-

epithelial tissue swells and keeps pace with the villi as they increase in length."

The form and relation of maternal and foetal vessels in the placenta of sheep is studied by Barcroft and Barron (1946). They observe that the arteries in the uterus extend towards the mucosal surface of the cotyledon and break up into capillaries which form a subepithelial plexus that is drained by capillaries and veins parallel to the arteries. The chorionic villi have central arteries which break up at the distal ends into superficial capillary nets which drain into veins at the bases of the villi. Therefore the blood flows in the opposite directions in parallel nets of maximum development early in gestation while the maternal vascular bed increases steadily. They notice that in the late part of gestation (about 131 days) the maternal vessels are almost devoid of stroma and their endothelial walls are in direct contact with the foetal tissue. Wimsatt (1950), on the other hand, investigating the nature of the placental barrier by special staining of reticular fibres, concludes that neither foetal nor maternal vessels are completely denuded.

The extravasation of blood has been reported by both Jenkinson (1906) and Assheton (1906). The latter noticed that the vessels on the crests of the maternal septa burst about the tenth week of pregnancy and give rise to lacunae of extravasated blood which bath the bases of the foetal villi. He found pigment in the chorionic cells at the bases of the villi and suggested that - "The fact of the

accumulation of pigment in the bases of the villi leads one to suspect that the cotyledonary areas are more concerned with excretion and possibly with respiration than with nutrition." Jenkinson (1906) also observed that the red blood corpuscles are ingested by the trophoblasts and the ingested corpuscles might include yellowish brown pigment or both corpuscles and pigments may be seen in one and the same cell. He did not get an iron reaction within these cells. Grant (1933) reported that the melanoblasts of the uterine mucosa are largely destroyed by the foetal trophoblasts during pregnancy. He thinks that no physiological significance is attributed to the pigment and the highly insoluble nature of the melanin renders the hypothesis that the pigment is utilised by the foetus improbable. Barcroft and Barron (1946) observed that in 45 days pregnancy the walls of many individual vessels are broken down and maternal blood is extravasated on the cotyledonary surface. Wimsatt (1950) presented histological evidence that the escape of blood is sporadic and it is believed to be effected by the necrotic collapse of the terminal portions of the maternal septa induced by a static vascular congestion in the maternal capillaries, rather than by the direct histolytic agency of the trophoblast.

Histochemical Findings

The uterus and placenta are the organs through which substances pass from the mother to the foetus for its nutrition and certain substances are produced in the latter, namely placental steroids. Therefore, these organs have

been studied histochemically to investigate the various compositions of their cells. Amoroso (1956) quotes - "The nutritive materials supplied to the foetus may reach it direct from the circulating blood in the placenta 'haemotroph' or through the absorption of products of the endometrium itself 'histotrophe' (Grosser, 1927)."

In the placenta of sheep Assheton (1906) is inclined to believe that the important part of nourishment of the embryo is derived from the secretion by the uterine glands and general uterine epithelium and suspects that the cotyledonary area is more concerned with excretion and possibly with respiration than with nutrition. Jenkinson (1906) in cow and sheep has reported that the secretion of fat by the uterine and glandular epithelium into the lumen is an apocrine secretion. He also described the presence and distribution of glycogen, in the sheep, in the subepithelial connective tissue and uterine milk as well as in the trophoblast and connective tissue of the chorion and allantoic epithelium but not in the villi. Wimsatt (1951) observed that the binucleate giant cell in the placenta of sheep and cow is highly polarised both morphologically and physiologically. He noticed that the binucleate cells are post-mitotic, i.e. incapable of cytokinesis, erythrophagocytic and rich in a variety of chemical materials including alkaline phosphatase and possibly acid phosphatase, ribonucleic acid and carbohydrate-protein complexes. In some parts of the chorion he found lipid droplets of variable size and number. Despite the presence of these

chemical materials, Wimsatt was against the suggestion that the binucleate cells might have a secretory function because there was no evidence of discharge ever observed outside the cells, the material in the cells was diffused and not granular and occupied the opposite pole from that in which the Golgi and mitochondrial bodies are segregated.

Fahmy (1953) in the sheep and goat found more alkaline phosphatase in the maternal septa than in the chorionic villi. In the sheep alkaline phosphatase activity was limited to the distal border of the binucleate cells and the cells covering the chorionic villi and those lining the maternal septa, but on the 140th day the enzymic activity was diminished in both foetal and maternal tissues, being confined to the binucleate cells in the chorionic villi and the endothelial lining of the maternal vessels. The surface and the glandular epithelia in the sheep and goat showed both phosphatase enzymes. In the maternal septa, acid phosphatase distribution was central, in contrast to alkaline phosphatase which took a peripheral distribution except near full term. Fahmy found no glycogen in the sheep's placenta by Best's method but the Bauer-Feulgen method stained the connective tissue of the maternal septa and chorionic villi, the binucleate cells and the subepithelial layer of the intercotyledonary areas. He found fine and faint glycogen granules in the surface epithelium using the Bauer-Feulgen method. Fahmy and Huggett (1954), on the same subject, conclude that glycogen is absent in the cotyledons of the sheep's placenta but

present in the intercotyledonary zone. Alkaline phosphatase is present mainly in the decidual part and slightly in the tips of the foetal villi, while in rodent and man it is mainly in the syncytiotrophoblast. Alkaline phosphatase and glycogen never appear together in the same cell; where one increases or decreases with placental aging, the other may disappear or appear in that cell.

Hamilton, Harrison and Young (1960) on placentation in certain cervidae describe the distribution of PAS positive material, alkaline phosphatase and lipids. They found in the fully differentiated villus that the main stem consists of tall columnar trophoblastic cells supported by a PAS positive basement membrane, the apices of the cells showing cytoplasmic protuberances containing PAS positive droplets mostly glycogen. Some of these droplets lie outside the cells and these increase as pregnancy advances. There is an abundant distribution of alkaline B-glycerophosphatase as a band along the outer surface of the columnar trophoblastic cells and there is some intracellular distribution within individual cells. Minute lipid droplets can be seen scattered throughout the cytoplasm in frozen sections. Binucleate cells which appear in the second zone next to the main stem villi have fine PAS positive granules distributed throughout their cytoplasm and they are also supported by a PAS positive basement membrane. Towards the tips of the villi the binucleate cells progressively contain more PAS positive material. One or more large lipid droplets are frequently seen in the cytoplasm. Alkaline phosphatase is

distributed evenly throughout the cytoplasm, but is scanty or lacking in the nuclei. Some binucleate cells, however, possess only sparse amounts of the enzyme. No maternal erythrocytes, non-ferruginous pigments, or strongly acidophilic proteinous crystals as described by Wimsatt (1951) have been identified. Alkaline phosphatase is distributed in a broad band along the region of contact between the trophoblast and the crypt lining. Many binucleate cells in the crypt lining have little or no PAS positive material. The tissue of the maternal septa consists of the crypt lining cells supported by a PAS positive basement membrane, stromal cells and a few fibroblasts. The endothelial cells are thin and contain alkaline phosphatase and a PAS positive membrane surrounds the endothelial lining. Hamilton et al. also state that - "The tips of the septa are necrotic ..... The material displays less and less PAS positivity towards the tip; some glycogen is present as droplets lying against the wall." Alkaline phosphatase is abundant in the deeper parts of the septa but is altogether absent in the necrotic tip region.

Hadek (1954-55) describes some histochemical aspects of the reproductive tract of sheep during the oestrous cycle. The secretory products in the sheep's oviduct are found to be acid mucopolysaccharide and most profuse at the time of ovulation. He claims that alkaline phosphatase in the oviduct shows cyclical alteration similar to acid mucopolysaccharide but according to his results no lipids

were encountered in the tubal epithelium. In the case of the uterine secretions he sums up that those substances which appear in pro-oestrous show an increase during oestrous and disappear thereafter, and those which appear during oestrous increase during metoestrous and disappear later. He found that iron belongs to the first group, lipids to the second and mucoprotein, alkaline phosphatase and ribonucleic acid occupy a phase between them. In other words, this indicates that the inorganic iron is influenced by the folliculin, while the lipid and mucoprotein are dependent on the progesterone secretion and especially ribonucleic acid and alkaline phosphatase which appear at the time of progesterone activity.

It should be noted that all the histochemical findings reviewed were based on chemically fixed material and frozen sections. From the literature cited it is also clear that uncertainty still remains regarding the nature of the cells lining the maternal crypts and intercrypt columns, at least in the sheep.

The morphology and histochemistry of the oviducts of sheep were inadequately studied especially during pregnancy. The characteristic picture of the tubal epithelium during pregnancy suggests a further investigation on the morphology and histochemistry of the oviducts during the oestrous cycle and pregnancy. The application of freeze drying and freeze substitution techniques should help in the preservation and localisation of the investigated substance. The ultrastructure of the tubal

epithelium might also throw some light on the morphology and function.

The morphology and histochemistry of the uterus and placenta of sheep have been studied by many authors but it is felt that a study of the histochemistry of these organs using the new techniques of freeze drying and freeze substitution would be of value. However, the findings with the electron microscope regarding the foetal-maternal relationship in the cow and deer raised anew the idea of the simple epithelio-chorial placenta according to Grosser's classification. This suggested that in the present study an examination of the fine structure of the junctional zone of the sheep's placenta should be carried out, in order to contribute to the knowledge of the foetal-maternal relationship in the sheep.

MATERIALS AND METHODS

The oviducts and uteri of 39 non-pregnant sheep and the oviducts, uteri and placentae of 54 pregnant sheep were used in this study. 14 of the specimens from the pregnant sheep group were of known history, i.e. killed at certain stages of pregnancy, between 40 and 140 days; these were procured from the Animal Diseases Research Association, Moredum Institute, Edinburgh. The rest of the material was collected from Edinburgh City Abattoir. The breeds of sheep were Black-faced, Suffolk and Leicester-Cheviot crosses.

Selection and classification of the specimens collected from non-pregnant animals were based on the valuable work of Quinlan and Mare (1931) and Grant (1933) who described in detail the ovarian changes and formation of the corpus luteum in the sheep during the oestrous cycle and pregnancy (Table I).

Foetuses from gravid uteri were measured crown/rump length. The estimation of foetal ages of specimens collected from the Abattoir was based on these measurements by comparing them with the measurements of foetuses of known ages from the Moredun Institute and with other historical data on the subject (Assheton, 1906, and Barcroft, 1952), (Table II).

The material collected was fixed or quenched as soon as possible but a delay of 20 - 30 minutes between the killing of the animal and the collection of material was inevitable. Small pieces of tissues from different parts

of the oviducts, uterus and placenta of each specimen were fixed in appropriate fixatives. Other smaller pieces of tissues from similar areas were treated by freeze drying and freeze substitution techniques as described below.

The chemically fixed tissues, after suitable time in the fixative, were dehydrated in alcohol, cleared in benzene or xylene and embedded in paraffin wax (56°C. m.p.). The tissues were preserved in different fixatives depending upon the subsequent techniques to be used. For general morphology both 10% neutral formalin and Bouin's fluid were used. Buffered 10% formalin at 4°C. was used for tissues to be stained for acid phosphatase and inorganic iron. For glycogen, mucin, ribonucleic acid and alkaline phosphatase, Lillie's fixative (alcohol acetic acid-formalin) at 4°C. was used. The period of fixation was usually 24 - 48 hours. Frozen sections were cut and stained for lipids but the controlled chromation method according to Elftman (1958) was found to give better results. This method depended upon the oxidation and fixation of lipids by an acidic potassium dichromate pH 2.5 at 60°C. for 18 hours after which the lipids could stand the procedure of dehydrating, clearing and embedding. The small pieces of chromated tissues, approximately 5 mm. in thickness, were transferred more rapidly than other tissues through the alcohols, xylene and paraffin wax (total time 3 hours).

The freeze drying and freeze substitution techniques were used to investigate especially enzyme activity but parallel tests for other substances were made to compare

with the results obtained in the chemically fixed tissues. The procedures of freeze drying and freeze substitution were carried out as follows:-

1. Small pieces of tissues, 3 - 5 mm., were cut from the specimens as soon as they were collected and put on strips of aluminium foil weighted with lead shot.
2. The fresh tissues were plunged quickly into isopentane cooled to about -170°C. with liquid nitrogen. It is essential for the tissue to be cooled as rapidly as possible.
3. The solid frozen tissues were removed from the quenching fluid and kept in dry ice until the freeze dryer was ready to receive them.
4. The freeze dryer is equipped with two vapour traps of P_2O_5, one inside the vacuum chamber in which the tissues are placed and the other immediately above the vacuum pump. The quenched tissues were placed in separate compartments of the vacuum chamber at -40°C. The vacuum chamber was then closed and a high vacuum created within it. After a number of trials, the tissues were found to dry in approximately 72 hours during which time the P_2O_5 in the second vapour trap was changed twice.
5. After drying, the tissues were transferred and embedded in polyester wax (39°C. m.p.).

Freeze substitution tissues were quenched in a similar way, then the solid frozen tissues were dropped into absolute alcohol at -40°C. to which mercuric chloride

had been added. The tissues were left for 48 hours, then they were embedded and blocked in polyester wax.

After blocking, the tissues treated by freeze drying and freeze substitution techniques were kept in a cold store until required for sectioning. Sections were cut at 5 - 8 mu. thickness and flattened on slides with a cold formol/calcium solution which acted as a fixative for freeze dried tissues.

The freeze drying technique was modified later as follows. The temperature of the drying chamber was raised to -25°C. and drying was completed within 24 - 36 hours time. The tissues were then embedded in soft paraffin wax (39°C. m.p.) instead of the water soluble polyester wax. Sections were cut in the usual way but they were flattened by floating on formol/calcium or absolute alcohol. The modified method preserved phosphatase enzymes and PAS positive substances better than the older technique, especially when the sections were flattened and fixed by absolute alcohol. Two sections from the same block, one flattened and fixed by absolute alcohol and the other by formol/calcium gave different results for acid phosphatase, i.e. the former gave a positive reaction while the latter gave little or no reaction. The freeze substitution tissues embedded in soft paraffin and floated out on absolute alcohol did not give a positive reaction for acid phosphatase. These results after the modification of techniques would indicate that both formalin and alcohol could inactivate acid

phosphatase to a certain extent, but inactivation by the latter is only apparent after long treatment even at low temperatures.

The chemical reactions and staining of tissues for different substances and structures were carried out by the following methods:-

1. Morphology - Haematoxylin and eosin as a routine technique. Masson's trichrome stain as a differential staining method. Wilder's silver oxide solution for reticular fibres.
2. Glycogen and Mucin - PAS technique (McManus) as routine technique. Best's carmine as a selective stain for glycogen. Diastase digestion was used as a control in both cases. Acidic alcian blue and toluidine blue for mucins.
3. Alkaline and Acid Phosphatases (Sodium B-glycero-phosphate substrate) - Calcium-cobalt method and lead nitrate method after Gomori were used respectively. The latter was modified according to Wachstein et al. (1962) by using a pH 6 instead of pH 4.9. The best results were obtained by freeze dried tissues embedded in soft paraffin and briefly fixed by absolute alcohol. Control sections were treated in a similar manner omitting the substrate.

4. Lipids - Sudan Black B in propylene glycol gave a better staining of lipid droplets than Sudan Black B in 70% alcohol or other Sudan stains. The controlled chromation method gave a better result than frozen sections. Acid haematin method (Baker's, 1946) was used as a parallel test to confirm the results obtained by the Sudan Black B method.

5. Nucleic Acids - Methyl Green-Pyronin Y method for DNA and RNA (after Kurnick, 1955) was used. Control sections were treated with 1N-HCl prior to staining for removal of both nucleic acids, while parallel sections were treated with 10% perchloric acid at 4°C. for 12 - 18 hours for removal of RNA alone (Erickson et al., 1949).

6. Inorganic Iron - Perl's method for ferric iron was used as recommended by Lillie (1954) and quoted by Pearse (1960). Tissues fixed in neutral formalin were recommended but in the present study freeze dried tissues also gave good results.

Stained sections were examined under the light microscope for general morphology and histochemical reactions. Phase contrast microscopy was used for examining unstained freeze dried sections and for sections stained for phosphatases.

For examination of the fine structure of the tubal epithelium and placentome under the electron microscope, sections were prepared as follows.

Fresh small pieces of tissues 3 mm. thick from a fully developed central placentome and the oviduct from the same specimen were fixed in cold glutaldehyde solution as soon as the specimen was collected in the slaughterhouse. In the laboratory, smaller pieces of 1 mm. thickness were selected and transferred to Palade's sucrose fixative. Fixation time 3 hours. The tissues were quickly dehydrated in alcohols, transferred to epoxypropane-Aruldite mixture and then embedded in fresh Aruldite. The polymorisation was carried out at 60°C. for 48 hours. The gelatin capsule was then removed and the block trimmed before section cutting. Sections were cut by an ultra microtome and placed in copper grids. The grids containing the sections were left to dry and then stained by lead citrate and uranyl acetate solutions.

TABLE I

Specimens from non-pregnant sheep collected during the different phases of the oestrous cycle and anoestrous

Phase of the oestrous cycle	No. of specimens collected	Condition of the ovaries
1. Pro-oestrous	12	Growing Graafian follicles 5-10 mm. in diameter; corpora lutea of previous cycle are present in most of the cases collected.
2. Metoestrous	5	Small developing corpora lutea, average diameter 6 mm., seen as a pinkish-red prominence; a small blood clot may be present indicating the point of rupture of the follicle.
3. Early Dioestrous	7	Developing corpora lutea, 8-10 mm. in diameter, appearing as dark-reddish rosette-shaped prominences.
4. Late Dioestrous	11	Fully developed corpora lutea, average diameter 10 mm., appearing spherical in shape and less prominent; their colour changes to pale reddish-pink.
Anoestrous	4	Small follicles and old corpora lutea are not uncommon. Specimens were collected during the resting season.

TABLE II

Specimens collected from pregnant sheep
C/R length measurements and estimation of foetal ages

C/R length	Foetal age	No. of specimens collected
5 mm.	less than 20 days	1
10-20 mm.	less than - 30 days	9
25-40 mm.	± 35 days	6
50-100 mm.	± 45 days	12
100-170 mm.	± 65 days	4
200-350 mm.	± 90 days	12
400-500+ mm.	± 140 days	10

OBSERVATIONS ON THE OVIDUCTS AND UTERUS OF THE SHEEP DURING THE OESTROUS CYCLE

The Oviducts

The oviduct of the sheep, similar to those of other domestic animals, has fimbiae at its abdominal end, the infundibulum, closely related to the ovary. The infundibulum leads into the ampulla, which has a wider diameter than the isthmus. The wall of the ampulla is flabby in consistency and its mucosa is extensively folded, forming primary and secondary folds. The former are longitudinal folds in transverse section while the latter extend deep into the lumen and branch in different directions. The tubal epithelium consists of ciliated and non-ciliated columnar cells which exhibit marked changes during the oestrous cycle. The lining epithelium is supported by a basement membrane and a subepithelial layer of connective tissue containing vessels and nerves.

The isthmus region is lined by simple columnar epithelium, and the mucosal folds are low and do not branch as in the ampulla. The subepithelial layer is cellular and is supported by a thick circular muscle layer interspersed by collagenous fibres continuous from the subepithelial layer. The thick circular muscle layer in the isthmus region makes this part of the tube hard in consistency. Melanoblasts are not uncommon in this layer (Fig. 1).

(The description which follows is always referring to the ampullary part of the oviduct except when otherwise indicated.)

During anoestrum, i.e. before and after the breeding season, the oviduct is in a quiescent stage. The tubal epithelium consists of regular columnar cells, most of which are ciliated. No secretory activity is noticed but remains of secretion usually persist at the free border of the cells. The subepithelial layer is narrow and consists of scanty connective tissue, small vessels and a few lymphocytes (Fig. 2).

At the beginning of the breeding season and when the follicles are growing, marked changes are noticed in the tubal epithelium. There is proliferation and increase in the height of the cells and in many places the epithelium looks pseudostratified. The mucosal folds appear swollen and the subepithelial layer consists of rather loose vascular connective tissue with hypertrophied cells. Towards the end of the pro-oestrous period, when the Graafian follicle is ripe and about to rupture, secretion is noticed in the lumen of the oviduct and on the free border of the cells. The subepithelial layer is vascular and hypertrophied (Fig. 3).

After the rupture of the follicle, i.e. during metoestrum and early dioestrum the tubal epithelium is in a secretory phase. Secretion is noticed within the cells as well as at the free border and in the lumen of the tube. The epithelium is very high and appears pseudostratified. Large cytoplasmic projections are found protruding from the free margin of some of the non-ciliated secreting cells. The subepithelial layer is very vascular and the vessels

are supported by loose connective tissue in which a few lymphocytes are encountered at the bases of the epithelial cells. The mucosal folds are swollen and tend to form short lateral branches (Fig. 4).

During the rest of the dioestrous period, although the tubal epithelium is less tall it appears pseudo-stratified in some places. The secretory activity is gradually lessened and the nuclei occupy different positions in the cells. The characteristic feature of the late dioestrous period is the irregular free surface of the epithelium due to the cytoplasmic projections from the cells and the migrating nuclei with tapering tails which are partly intra-cellular. This condition most probably indicates degenerative changes in the exhausted secretory cells. Infiltration of lymphocytes is increased in the subepithelial layer and tubal epithelium. The mucosal folds become less swollen and less vascular (Fig. 5).

Before a period of anoestrum is established in the tubal mucosa, proliferative changes for the next pro-oestrous period are apparent and similar changes to those described above are repeated in the succeeding stages.

Summing up the changes in the tubal mucosa of the sheep during the oestrous cycle: the tubal epithelium in pro-oestrum is in a phase of growth and in post-oestrum is in a phase of secretory activity which gradually diminishes towards the next pro-oestrum when another period of growth begins again.

Changes in the isthmus region are less marked than in the ampullary region and the secretory activity is

negligible. The tubal epithelium in the isthmus is always of simple columnar type but there is an increase in the height of the cells during the growth period but this is not followed by secretory activity as noticed in the ampullary part of the oviduct.

The Uterus

The uterine epithelium during anoestrum is simple columnar with elongated nuclei occupying the basal two thirds of the cells. The epithelial cells are supported by a wide basement membrane and the subepithelial layer appears cellular with a few collagenous fibres, blood vessels and nerves. In the intercotyledonary areas the ducts of the uterine glands are lined by an epithelium, similar to that of the uterine surface and continuous with it, at the openings of the glands. The secretory parts of the glands are lined by low columnar epithelial cells and have narrow lumina. There is infiltration of lymphocytes around the glands and at the bases of the uterine epithelium as well as in the stroma (Fig. 6). The subepithelial layer in the caruncular areas is more cellular and contains many blood vessels. Melanoblasts are encountered near the epithelial lining in some of the specimens examined. Apart from the melanoblasts there are large round cells with spherical nuclei and brown-yellowish cytoplasm. These round cells have certain chemical properties (see histochemistry).

In the breeding season and during the growth of the follicle, i.e. pro-oestrum, the most marked change in the uterus is the increase in the height of the epithelial

cells both in the uterine and glandular epithelium (Fig. 7). This is preceded by an increase in vascularisation of the endometrial stroma; here the blood vessels increase in number and run parallel towards the surface of the uterus. This is especially marked in the caruncular areas where the subepithelial layer is very thick and cellular. In the deeper parts of the caruncular areas the endometrial stroma becomes loose connective tissue supporting the blood vessels and secretory parts of the glands which continue with those in the intercaruncular areas. A few lymphocytes are still encountered at the bases of the epithelial cells and in stromal tissue but they seem fewer than in other stages of the cycle. In some of the specimens melanoblasts are encountered in the subepithelial layer.

During metoestrum, i.e. after the rupture of the Graafian follicle, the uterus shows much activity. The surface epithelium is tall columnar and in some places appears pseudostratified columnar. The endometrial stroma is hypertrophied and the blood vessels are congested. The glandular tissues look active and secretion is noticed at the necks and openings of glands. The secretory parts are lined by columnar cells and their nuclei are situated towards the bases of the cells. The uterine glands appear long and convoluted. Infiltration of lymphocytes reappear at the bases and inside some of the epithelial cells and in the stromal tissue (Fig. 8).

At early dioestrum the picture is more or less similar to that of metoestrum with more secretion in the

lumen of the uterus, and the surface epithelium appears to be more pseudostratified. The endometrial stroma remains cellular and vascular at the caruncular areas and the uterine glands are in secretory or excretory phases. Lymphocytic infiltration is noticeable around glands and in the subepithelial layer; some lymphocytes are intra-epithelial. In the intercotyledonary areas the mucosa appears more folded than at earlier stages of the cycle (Fig. 9).

Towards the late part of dioestrum there is a general retardation of the uterine activity. An increase in the lymphocytic infiltration is noticeable. Nevertheless, the endometrium does not regress to the extent of that seen during anoestrum. Towards the end of the cycle the condition of the endometrium either resembles that found in the dioestrum or early pro-oestrum. This is probably due to the short period of the oestrous cycle in the sheep (16 days approximately).

Summing up the endometrial changes during the oestrous cycle: pro-oestrum is a period of growth of epithelial and stromal tissues (caruncular areas are cellular and vascular and intercaruncular areas are glandular); this is preceded by increased vascularisation. Metoestrum and early dioestrum are periods of activity in growth and secretion (epithelium is pseudostratified and glands are long and convoluted). Finally, late dioestrum is a period of regression and infiltration of leucocytes which is cut short by the growth period of the next cycle.

Histochemistry

Oviducts

Carbohydrates - PAS material (removable by diastase and brilliantly stained by Best's carmine) is found in minute and large granules in the cytoplasmic projections on the distal border of the tubal epithelium. These granules are noticed during late dioestrum - pro-oestrum periods but are minimal or absent during metoestrum - dioestrum periods. Some of these granules are noticed at the bases of the epithelial cells and in the subepithelial layer.

PAS positive material (diastase resistant, stained by Alcian blue and B-metachromatic) is found in the tubal epithelium but varies in quantity and in location according to the different stages of the oestrous cycle. The PAS positive material appears inside the cells (supra-nuclear) during pro-oestrum. By late pro-oestrum and onset of metoestrum the material is found outside and at the borders of the cells (Figs. 10 and 11). During dioestrum and until the beginning of the new proliferative phase (pro-oestrum) the material is noticed at the free border of the cells and in the cytoplasmic projections characteristic of this period (Fig. 5). This material is also found in the secretion in the lumen of the tube with free nuclei which have migrated from the tubal epithelium or the underlying connective tissue.

Acid and Alkaline B-glycerophosphatases - The activity of alkaline B-glycerophosphatase is noticed along the free border of the tubal epithelium. The ampulla

shows less enzymic activity than the isthmus which is adjoining the uterine horn. The activity is limited to a narrow zone at the border of the cells and the cilia of ciliated cells (Fig. 12). The enzymic activity is observed in the secretion in the lumen of the tube between the mucosal folds. During the cycle alkaline phosphatase is invariably present at the described sites but the activity tends to increase during metoestrum and dioestrum (Figs. 13 and 14).

The acid B-glycerophosphatase activity is also observed in the tubal epithelium and in the supranuclear parts of the non-ciliated cells (Figs. 15 and 16). The activity is more apparent in pro-oestrum and metoestrum. This is clearly shown in freeze-dried sections while in the formalin fixed sections much of the activity is lost (possibly by inactivation during the processing) and the activity which persists is less than that of alkaline phosphatase. In the activities of both enzymes the reaction in the nuclei is regarded as an artifact and therefore not considered.

Lipids - Lipid droplets (Sudanophilic) are found in the tubal epithelium as large and small droplets usually in the supranuclear parts of the cells, especially in the Golgi zone. Lipid droplets are present in the tubal epithelium throughout the oestrous cycle. Though this is not a rule, it is noticeable that these droplets tend to be of irregular sizes and scattered in the cells during late dioestrum - pro-oestrum and appear more concentrated at the

Golgi zone during metoestrum - dioestrum. Lipid droplets are also noticed in the subepithelial layer and in the muscle layers but they are scattered in no particular arrangement and in less concentration (Fig. 17).

At the isthmus region lipid droplets invariably occupy the Golgi zone in the epithelial cells and do not show changes as in the ampullary region. Lipid droplets are also present in other layers of the wall of the oviduct (Fig. 18).

Ribonucleic Acid - Ribonucleic acid is found in the tubal epithelium in varying concentrations according to the different phases of the oestrous cycle. It is noticed as intracellular and evenly distributed in the cells during pro-oestrum. Towards late pro-oestrum it occupies a supranuclear position and appears partly outside the cells in the cytoplasmic projections. In post-oestrum periods ribonucleic acid appears in less concentrations in the distal part of the cells and cytoplasmic projections (Fig. 19).

Iron - The presence of inorganic iron in the tubal epithelium has been detected by Perl's method and the reaction is found to be limited to the distal border of the cells especially at the cilia. Inside the cells a few minute granules irregular in size and distribution are encountered. The inner lining of the blood vessels in the subepithelial layer reacted positively to the iron test and some granules appeared scattered in the connective tissue of the propria. Traces of iron granules in the tubal mucosa are noticed throughout the oestrous cycle (Fig. 20).

Uterus

Carbohydrates - No glycogen is encountered in the uterus of non-pregnant sheep. A few irregular granules which are noticed in some of the secretory parts of the uterine glands are not constant and sometimes appear extracellular (probably staining artifact).

PAS material resistant to diastase is observed along the distal border of the uterine epithelium and the cells lining the openings of the glands. In some specimens this material is noticed partly in the lumen near the border of the cells. Similar material is always present in the basement membrane of the surface epithelium and in the inner walls of the blood vessels of the endometrial stroma. The PAS material diastase resistant is also noticed in the large round cells scattered in the endometrial stroma (Fig. 21). These cells appear in sections stained by Haematoxylin and eosin as large round cells with dark staining nuclei and brown-yellowish cytoplasm.

The inner walls of the endometrial blood vessels stained lightly with Alcian blue and were negative to Best's carmine staining. The border of the surface epithelium and that lining the necks of the uterine glands stained well with Alcian blue but stained faintly by Best's carmine. The round cells in the stroma also stained by Alcian blue and faintly by Best's carmine.

At metoestrum and early dioestrum PAS material diastase resistant is noticed in the secretion products in the uterine lumen and at the openings of the glands but is

never noticed within the glandular or uterine epithelial cells. This material is possibly a mucoprotein or glycoprotein, i.e. a carbohydrate-protein complex (Pearse, 1960).

Phosphatases - The enzyme activity of alkaline and acid phosphatases is observed in the uterus of non-pregnant sheep. The localisation of the activity of both enzymes is more or less similar, i.e. at the distal border of the uterine and glandular epithelia. Alkaline phosphatase activity is found in the glands and surface epithelium near the free surface of the cells and within the lumen. Activity is also noticed in the lining of the superficial blood vessels especially during metoestrum and early dioestrum (Fig. 22). The enzyme activity seems to be directly under the control of the corpus luteum activity.

Acid phosphatase activity has a similar distribution and in similar periods but it is not commonly seen in the lining of the blood vessels. It appears more concentrated in the lumen of the uterine glands than at the border of the cells (Fig. 23). Activity is also noticed, but very faint in the blood erythrocytes.

It is clear from the reaction in the lumen that both enzymes are apparently active outside the cells or at their plasmal membranes. It is also possible that the enzymes are secreted by the glandular epithelia and become active thereafter. During pregnancy acid phosphatase activity is noticed to be intracellular (see phosphatases of oviduct and uterus in pregnancy).

Lipids - Lipid droplets are always found in the uterus of non-pregnant sheep. They are observed in the

uterine glands and the uterine epithelium, especially in the supranuclear parts of the cells. During pro-oestrum lipid droplets are found both above and below the nuclei. In the endometrial stroma the droplets are seen scattered indiscriminately (Fig. 24).

During the oestrous cycle the lipid droplets are found in maximal concentration in the cells during pro-oestrum, they become less concentrated towards post-oestrum, and start to increase again during pro-oestrum. In late dioestrum and early pro-oestrum only a few droplets are noticed in the uterine epithelium and those found in the glandular epithelium are supranuclear in position and smaller in size than those noticed during metoestrum.

Ribonucleic Acid - It is found in the uterine glands in varying degrees of intensity during the oestrous cycle. It is constantly found in the uterine epithelium but lightly stained. During pro-oestrum the surface epithelium and the deeper parts of the glands show nucleic acid in the distal part of the cells while the superficial glandular tissue shows little or no nucleic acid. During metoestrum the quantity of nucleic acid increases in both uterine epithelium and glands. At dioestrum nucleic acid is present but gradually decreases in quantity, especially in the deeper regions of the glands. The lymphocyte-like cells and the round cells in the endometrial stroma also contain ribonucleic acid (Fig. 25).

Iron - Inorganic iron by Prussian blue method is found at the distal borders of the uterine epithelium and

in some of the secretory parts of the uterine glands (Fig. 26). The intensity of the reaction is variable in the epithelial tissue but it is most intense during pro-oestrum. In post-oestrous periods the reaction is gradually decreasing towards the end of the cycle. The inner lining of the superficial vessels gives a positive reaction but the reaction in the stromal tissue is diffused and it is considered non-specific.

OBSERVATIONS ON THE OVIDUCTS, UTERUS AND PLACENTA OF THE SHEEP DURING PREGNANCY

The Oviducts

During the first month of pregnancy the tubal epithelium in the infundibulum and ampullary region of the oviduct has an irregular free surface. Many of the nuclei of the non-ciliated secretory cells are protruding above the free surface of the cells while other cells with elongated nuclei seem to be replacing them. The protruding nuclei appear pear-shaped with the tapering portion partly intracellular (Fig. 27); some are enveloped by their secretion or cytoplasm while others have already left their cells and are free in the lumen. A few epithelial cells show cytoplasmic projections or large droplets of secretion with no nuclei. Other tubal epithelial cells are tall columnar ciliated cells with elongated nuclei; these are found below the degenerating cells described above. The mucous membrane is fairly vascular and its folds are swollen with loose connective tissue.

The isthmus part of the oviduct shows little change. The lining epithelium is tall columnar partly ciliated, the cilia are lost towards the uterine part of the tube, and no sign of activity or degeneration is apparent. The mucosa is less folded and fairly vascular and in some of the specimens the muscularis is infiltrated with pigment cells, especially the inner muscle layer.

During the second month of pregnancy the tubal epithelium continues to show a similar picture to that

described above with little variation. In the first half of the month many of the protruding nuclei disappear from the surface leaving the cytoplasmic projections attached to the cells, but during the second half of the month the protruding nuclei appear again in large numbers as if all the older cells have been replaced by new cells. These changes are apparent only in the ampullary region of the tube but no change is noticed in the isthmus.

Between the 45th day and 80th day of pregnancy the condition of the oviduct in the ampullary region remains more or less the same. The common feature of the tubal epithelium is its irregular surface due to the protrusion of nuclei and finger-like projections of the cytoplasm, with or without nuclei, which seem to be alternating with the other cells (Fig. 28). In fact, due to the presence of a continuous regular layer of simple columnar epithelium beneath the numerous protruding bodies, the picture gives the impression that the tubal epithelium is stratified.

During the second half of pregnancy the protruding nuclei disappear and only a few cytoplasmic projections can be seen. Meanwhile, the epithelium regains its normal surface, and some of the cells are ciliated while others remain non-ciliated. The mucosa is still fairly vascular but the folds are not swollen and branched as in the earlier stage of pregnancy. The condition of the oviduct continues to be rather inactive, up to the end of the fourth month of pregnancy.

About the 140th day of pregnancy and onwards till full term, cytoplasmic projections and protrusion of nuclei reappear but not so pronounced as in the previous stages of gestation (first half of pregnancy). Histochemical reactions show that the non-ciliated cells are secreting at this stage. The mucosa is rather vascular and many of the small vessels are congested. No change is noticeable in the tubal epithelium of the isthmus.

Summing up the general structure of the oviduct of pregnant sheep: the epithelium in the infundibulum and ampullary regions shows finger-like cytoplasmic projections and many extruded nuclei which appear above the surface of the simple columnar cells thus forming what appears to be a second layer. This picture is common during the first half of gestation and the histochemical reactions show it to be most probably a degeneration rather than a process of secretion. Moreover, during the second half of gestation the protruding bodies appear less and less until near full term when a new phase of secretory activity reappears in the tubal cells with increased vascularity of the mucosa.

The fine structure of the tubal epithelium of pregnant sheep as seen under the electron microscope shows ciliated and non-ciliated columnar cells. The non-ciliated cells contain in their cytoplasm mitochondria, vesicles and electron dense granules (Fig. 67). Under the low power of the electron microscope the tubal epithelium appears to be pseudo-stratified in some places.

Groups of ciliated cells alternate with groups of non-ciliated cells (Fig. 68).

The non-ciliated cells show apical cytoplasmic masses covered by plasmal membranes and sometimes contain nuclei. They protrude into the lumen of the tube, become separated and are found free in the lumen and even there they contain electron dense granules, vesicles and endoplasmic reticulum (Fig. 69). Microvilli are not numerous in the non-ciliated cells but irregular protrusions are noticed (Fig. 70).

The ciliated cells do not show electron dense granules but small vesicles are noticed in their cytoplasm. They are characterised by their numerous cilia which have basal corpuscles within the apical part of the cells. The cilium consists of nine peripheral and two central dark bands - the typical structure of cilia (Fig. 71). In addition to the cilia, microvilli are present; they are smaller in diameter than the cilia and are continuous with the plasmal membrane of their cells (Fig. 72).

The Uterus and Placenta

During the early stages of pregnancy and before implantation the uterine wall is lined by a tall columnar epithelium and the cells are hypertrophied with elongated nuclei towards their bases (Fig. 29). The ducts of the uterine glands are wide and lined by a similar epithelium to that of the uterine surface. The secretory parts of the glands are lined by a low columnar epithelium and supported by a vascular bed. The mucosa in the inter-

cotyledonary areas consists of loose connective tissue supporting the vessels. The mucosa of the caruncular areas looks cellular and vascular, and many small vessels are seen running at right angles to the surface.

The smallest embryo recovered in the uterine cavity, in the specimens collected, measured 5 mm. The blastocyst is found occupying a small part of the uterine cavity, and the uterine epithelium even at this early stage of pregnancy is denuded at the sites of contact with the blastocyst, but elsewhere it is intact and tends to be pseudostratified in some places. The subepithelial layer is vascular and infiltrated with lymphocytes. The lymphocytes in the caruncular areas migrate towards the surface and in the intercaruncular areas they are found around the glands, some aggregates being noticed in the lumina of the glands (Fig. 30). Near the denuded surface of the caruncles, melanoblasts appear to migrate into the lumen. The endometrium in general looks swollen due to the hypertrophied glands and blood vessels.

In the gravid uteri which contain embryos of 14 - 20 mm. C.R.L., the uterine surface is denuded of lining epithelium in most places at the caruncular and intercaruncular areas in contact with the blastocyst. The lining epithelium is intact in the upper part of the non-gravid horn and even in many places in the tapering end of the gravid horn. Where it is present it is very high and in places is infiltrated by lymphocytes (Fig. 31). The endometrial stroma is vascular and looks oedematous; it is

infiltrated by lymphocytes especially around the glands and near the surface. Lymphocytes are also noticed in the lumina of the glands. Among the intact epithelium some lymphocyte-like cells which contain eosinophilic granules are encountered. The granules occupy opposite poles in the cells. These granules vary in size and quantity in each cell and are absent in others (Fig. 32).

The formation and development of the maternal crypts and intercrypt columns (maternal septa) is believed to have started at this stage of gestation. The caruncular areas appear denuded of lining epithelium. Only strands of foetal cells which may include binucleate giant cells are encountered here and there near the naked surface of the caruncles. The endometrial stroma in these areas is very cellular and vascular and tends to be folded in some places forming an undulated surface. Debris of degenerative cells is found in the uterine lumen.

When the embryo reaches the length of 25 mm. (approx. 30 days) the caruncles are denuded of covering epithelium and the cellular mucosa shows high ridges, the future maternal septa, which seem to have escaped the attack of the invasive chorionic cells. Between the ridges or columns of the caruncles there appear tracks of degenerative cells which are believed to be of maternal origin (Fig. 33). These degenerative cells eventually disappear thus giving rise to the furrows which will be occupied by the foetal villi. Before the movement of the foetal villi into the furrows, dark staining binucleate

cells are noticed at the surface of the maternal septa and between the mucosal folds. A few of these cells on the surface of the naked endometrial tissue are multi-nuclear while others are binucleate and occupy similar positions (Fig. 34). The intercaruncular areas are also denuded of lining epithelium and the endometrial stroma is oedematous and its blood vessels are congested. The glands are hypertrophied and their cells contain large vacuoles. Lymphocytes are seen migrating into the lumina of glands and towards the uterine surface. Lymphocyte-like cells containing granules are again noticed among the uterine epithelial cells. The lining epithelium is intact near the openings of the glands, at the junctions of caruncular and intercaruncular parts, and in many places at the tapering parts of the horns. At this stage where the uterine epithelium has degenerated and disappeared, replacement of cells can be seen at certain areas by the binucleate cells of the trophoblast and other giant multi-nucleate cells believed to be derived from them (Fig. 35).

When the embryo is 50 - 60 mm. C.R.L. (40 days approx.) the contact between the foetal and maternal tissues is becoming organised in the caruncular areas. The chorionic villi have grown deep into the furrows between the mucosal folds which form the maternal septa and thus giving the morphological picture of a simple placentome. The foetal villus is formed by loose vascular embryonic connective tissue lined by the chorionic epithelium which consists of tall columnar cells near the base and large

spherical or polygonal cells at the sides and near the tips. Among the polygonal cells are binucleate cells with dark staining nuclei. The nuclei of many of the binucleate cells at the free surface of the chorion appear eccentric and occupy the apical part of the cells. The maternal septum, on the other hand, is characterised by its dense vascular stromal tissue and its thin syncytial lining of coarse dark staining flattened nuclei and scarce cytoplasm. The lining of the maternal septa is interrupted in many places and near the bases of the septa consists of large giant cells. These are the multi-nucleated cells which have dark staining nuclei and appear to be of foetal origin rather than maternal. They are not continuous and therefore in some places the stroma looks naked (Fig. 36). Binucleate cells are also noticed among the multi-nucleate giant cells lining the maternal crypts. The nuclei of these cells maintain their apical eccentric positions. The histochemical observations support the view that binucleate cells give rise to the multi-nucleate giant cells (see histochemical findings).

In the intercaruncular areas the chorionic epithelium consists mainly of tall columnar cells among which binucleate cells are also found. A few of the latter are noticed in the space between the foetal and maternal tissues. The chorionic epithelium forms pocket-like invaginations or depressions, known in the pig as "areolae"; these usually correspond to the openings of the uterine glands (Fig. 37). In the maternal tissues hyper-

trophied glands and vascular connective tissue are the characteristic features, and the uterine epithelium seems to have regenerated from that at the openings of the glands. Nevertheless in some places the surface of the endometrium is lined by a thin syncytial lining with dark staining nuclei. The uterine epithelium covering the sides of the placentomes is always found intact as well as that lining the tips of the uterine horns. Lymphocytic infiltration is noticed in the endometrial stroma, especially around the glands and in the glandular and uterine epithelium. Lymphocyte-like cells containing granules are also encountered in the uterine epithelium where it is intact or has regenerated.

When the foetus is 16 cm. C.R.L. (60 days approx.) there is a general growth of the placenta, especially the central placentomes which have grown very big and complex. The foetal villi become very long and send branches radially into the placentome. They appear quite swollen by the embryonic connective tissue. The binucleate cells are concentrated at the tips of the villi, and a few are noticed at the sides and at the bases. The multi-nucleate giant cells are lining the fundus of the maternal crypts but the septa are lined by the syncytial dark staining nuclei. The septa look slender but vascular. At the periphery of the placentome, i.e. at the sides of the caruncle, the uterine epithelium is present as interrupted lining but becomes continuous as it emerges with that of the intercaruncular areas. In the intercotyledonary areas the

epithelium is intact in many places and the uterine glands look hypertrophied and actively secreting. Lymphocytic infiltration is present in the stroma and the glandular and uterine epithelium. Granular lymphocyte-like cells are more apparent in both glandular and surface epithelium. The granular intra-epithelial cells seem to have increased in number and size. At the tips of the horns the epithelium is intact and looks folded. The chorionic epithelium at this region is necrotic and copious secretion is present in the uterine lumen.

When the foetus grows to the length of 20 - 30 cm. (80 - 90 days approx.) the placenta is fully differentiated and characterises the full-grown placenta of the ewe. The placentomes are very large and vascular. Extravasation of blood is noticed in many places along the bases of the villi. The bases of the villi are lined by tall columnar epithelium which looks phagocytic and contain many pigment granules and red blood corpuscles (Fig. 38). The villi are less swollen and are lined by columnar chorionic cells, which change to cubical or polygonal cells and contain among them spherical binucleate cells, especially towards their tips. The maternal septa are long and slender and form bud-like branches containing blood vessels (Fig. 39). The maternal tissue consists mainly of vascular tissue, lined by an interrupted syncytium containing dark staining flattened nuclei. The fundus of the maternal crypts is lined by multi-nucleated giant cells.

The picture at the tips of the horns shows hypertrophy of the uterine epithelium and glands, and signs of active

secretion (Fig. 40). The chorionic epithelium at this part of the uterine horn appears necrotic, pycnotic nuclei are present and there is a large amount of secretion in the lumen (Fig. 41). The intercaruncular areas show intact uterine epithelium and active uterine glands. Lymphocytic infiltration is noticed in the stroma and the lining epithelium. Intraepithelial granular cells are present among the surface epithelial cells.

True pigment cells were noticed in the subepithelial layer of some of the specimens examined, especially in the caruncular areas. The movement of these pigment cells could not be followed throughout the period of gestation because they are not invariably present in the specimens examined. However, from the observations made in a few of the specimens containing the melanin cells, it is believed that they undergo a sort of degeneration or autolysis and become phagocytosed by the trophoblast (Fig. 42).

During late pregnancy, when the foetus grows to 40 - 50 cm. C.R.L. (120 - 140 days up to full term), the picture of the placentomes does not change much from that described in the previous stage apart from the extravasation of blood which becomes more marked. The columnar phagocytic cells of the trophoblast contain more erythrocytes and pigment granules (Fig. 43). A general decrease in the size of the placentomes is noticeable and accordingly the foetal villi, although keeping their complexity of branching, become very slender compared to previous stages. The maternal septa are thin but very

vascular and all the small vessels are enveloped by dark staining syncytium. The fundus of the maternal crypts is lined by a similar lining which is interrupted in places. The binucleate cells are becoming fewer in number but are still present at the sides and towards the tips of the villi.

The nature of the placental barrier and the extent of denudation of the maternal tissues have been investigated by special staining of the reticular tissue and it is found that the small vessels and capillaries of the maternal septa are covered and separated from the syncytial lining by a thin reticulum or intercellular cement (Fig. 44). This may correspond to the intervening space between the binucleate cells and the endothelial lining of the capillaries as seen under the electron microscope.

The intercaruncular areas are still lined by tall columnar uterine epithelium, glands are very active and their lumina are large and contain secretion and debris. The maternal stroma, i.e. the connective tissue supporting the glands, is vascular, loose, and in places oedematous. Infiltration of lymphocytes is noticed in the stroma and lining epithelium and intraepithelial granular cells are also present. The chorionic epithelium in the inter-caruncular areas retains its columnar shape and contains among its cells the dark staining binucleate cells, and the embryonic connective tissue is very vascular.

Summing up the changes in the uterus and the development of the placentomes during pregnancy: during

early pregnancy there is a rapid growth of the blastocyst and the uterus. When the blastocyst comes in contact with the maternal tissue the uterine epithelium is destroyed in both caruncular and intercaruncular areas. The uterine epithelium remains intact at the openings of the glands and over the small areas at the junction of the caruncular and intercaruncular areas and at the tapering ends of the uterine horns which the expanding blastocyst does not contact. In the caruncular areas destruction of tissues is further noticed in some parts of the endometrium in the form of tracks at right angles to the surface of the caruncles. The chorionic epithelium looks folded and contains among its tall columnar cells, spherical bi-nucleate cells.

During the seventh week of pregnancy the contact between the foetal and maternal tissues is more established in the caruncular areas. The chorionic folds move deep into the furrows of the caruncles which appear as a result of the destruction and disappearance of stromal tissue. On the other hand, the remaining healthy tissue of the stroma seems to grow and increase thus moving into the spaces between the chorionic folds or villi. Thus the formation of foetal villi and maternal crypts become apparent.

As pregnancy advances, the chorionic folds and the maternal ridges grow in height and tend to branch and so give rise to the definitive foetal villi and maternal septa. The important changes noticed in this respect are in the

cells lining or covering the foetal villi and maternal septa. The former are covered by chorionic epithelium which is tall columnar at the bases of the villi and becomes low columnar or somewhat cuboidal towards the middle and distal ends of the villi. Binucleate cells are found among the chorionic epithelium lining the sides and the tips of the villi, they are predominant towards the tips and a few are noticed in the spaces between the villi and the crypts. The maternal septa are lined by dark staining cells which have scanty cytoplasm and they form a syncytium in many places. The fundus of the crypt is lined by large multinucleated and a few binucleated giant cells which are believed to form a syncytium in some places, especially when they come to lie over the surface of the maternal septa.

When the placenta is fully developed the villi are differentiated into long slender columns which give off radial branches. They are lined by a single layer of chorionic cells and their cores consist of scanty embryonic connective tissue and thin walled blood vessels. The binucleate cells are found towards the tips of the villi as well as at the sides, but they are generally less numerous than at earlier stages of gestation. The maternal septa also become long and slender and are lined by cells with dark staining nuclei; these cells look cuboidal in some places and syncytial in others. The septa consist mainly of vascular tissue and stromal cells which may include pigment cells and large giant cells. The blood vessels in

the maternal septa give rise to lateral branches which are directly enveloped by the dark staining lining cells. The endothelial lining is markedly thick. The tips of the septa look necrotic in many places and some of the vessels lie naked without covering epithelium and eventually some of the vessels may rupture so that extravasated blood is seen in the space between these necrotic tips of the septa and the bases of the villi. The chorionic cells in this region are phagocytic and contain blood cells and pigment granules.

The fundi of the maternal crypts are lined by large multinucleated or syncytial giant cells but the syncytial cells become flattened thus lining bigger areas and extending to the sides of the crypts. The maternal vessels run at right angles to the bases of the crypts and come to lie near the crypt lining in many places. Free binucleate cells are encountered here and there in the spaces between the tips of the villi and the maternal crypts. Debris of degenerating cells and pycnotic nuclei are also found in the spaces between the chorionic and maternal lining.

In the intercaruncular areas the chorionic epithelium consists of tall columnar cells among which binucleate cells are encountered. Near the opening of the uterine glands the chorionic epithelium is invaginated and gives rise to depressions similar to the areolae in the sow's placenta. Some of these areolae contain uterine milk and debris. The denuded surface of the endometrium appears to have regenerated after the second month of pregnancy. In

the fully developed placenta the foetal and maternal epithelia are seen in apposition to each other but at the opening of the uterine glands areolae are found.

The uterine glands are hypertrophied and active throughout pregnancy. During the second half of gestation the lumina of the glands are very wide and the glandular cells are actively secreting, showing protruberances at their free borders. The whole endometrial tissues are hypertrophied and vascular. At the tapering parts of the uterine horns the foetal tissue looks necrotic but the maternal tissue is quite healthy. The surface epithelium is high columnar and appears folded due to the growing stromal tissue. The glands are actively secreting and the endometrial stroma is vascular but consists of loose connective tissue and appears oedematous especially in the glandular regions. Copious secretion is present in the uterine lumen and usually degenerating cells are encountered in the secretion. Lymphocytic infiltration of the stroma and the glandular and surface epithelia is always present and sometimes lymphocytes are found in the lumina of glands and uterus. Granular lymphocyte-like cells are encountered among the glandular and surface epithelia; these granules are eosinophilic (see histochemical findings). The granular lymphocyte-like cells tend to increase in number and size as pregnancy advances. Melanoblasts and other spherical cells with brownish yellow cytoplasm are found near the stromal surface in some of the specimens examined.

Fine Structure of the Junctional Zone

Observations on the fine structure of the placental membrane of sheep (270 mm. C.R. foetal length - 90th day of gestation approx.) were made with special reference to the lining of the maternal crypts at the junctional zone with the trophoblast. The electron microscopic findings were as follows.

In a fully differentiated placentome the junctional zone of the foetal and maternal lining cells is characterised by the interdigitations of microvilli except where debris and electron dense secretory granules intervene. The chorionic cells show characteristic vesicles which are apical in position and sometimes seen near the microvilli (Fig. 73). The cells lining the maternal tissue also possess vesicles which are fewer and smaller than those in the foetal cells but their apical cytoplasm is characterised by electron dense granules. These dense granules are also noticed in the spaces between the foetal and maternal tissues (Fig. 74).

The binucleate cells found in the lining of the maternal side possess only short microvilli when they are in direct contact with the trophoblast while the neighbouring cells show long microvilli which interdigitate with the chorionic cells (Fig. 75). On the foetal side the binucleate cells do not have microvilli at all and they appear partly covered by a process from a neighbouring cell, and the intercellular membrane is smooth and unfolded (Figs. 76 and 77). The cytoplasm of the binucleate cells contains

many electron dense granules, some vesicles and endoplasmic reticulum. It generally appears more dense than that of neighbouring cells; their nuclei also appear more electron dense than the nuclei of other cells but resemble to some extent those of the syncytial cells. The basal border of the binucleate cell is usually near a maternal capillary and is separated from the latter by a relatively wide intervening space. The plasma membrane of the binucleate cell shows infoldings which probably indicate pinocytosis (Fig. 74).

On comparison of a foetal capillary with a maternal capillary, the latter always has a thicker endothelial lining and a wider intervening space than the former. Both capillaries show tongue-like protrusions from the endothelial lining into the lumen. Mitochondria are present on both foetal and maternal lining cells and occupy a supranuclear position in the cells. The mitochondria on the foetal side appear bigger in size than those of the maternal side. Endoplasmic reticulum is also noticed in both foetal and maternal epithelia as well as in the binucleate cells. Along the lining of the endoplasmic reticulum minute electron dense granules are noticed which probably indicate ribose granules (Fig. 78).

The syncytial lining of the maternal septa is clearly seen in some places and no plasmal membrane separates the electron dense nuclei which are included in the syncytium (Fig. 79). These syncytial cells also possess microvilli, vesicles and electron dense granules and mitochondria. In

some areas where secretion and debris intervene, the foetal microvilli do not interdigitate with the maternal lining membranes and in some cases no microvilli are present in the maternal lining cells.

Histochemistry

Oviducts of Pregnant Sheep

Glycogen and Mucin - PAS positive material removable by diastase and stainable by Best's carmine (glycogen) is found in minute granules in the finger-like cytoplasmic projections on the free border of the tubal cells. Some granules are found deep within the tubal cells, these granules varying in size and quantity during gestation (Fig. 45). Only a small quantity of glycogen appears during the first month of pregnancy and it is mainly located at the free border of the tubal epithelial cells. About the 40th day of pregnancy, glycogen granules show a marked increase within the cytoplasmic projections and the tubal epithelial cells. The glycogen granules in the deep part of the cells are not constant and seen only in some of the specimens during this period of gestation. The granules continue to be present during mid-pregnancy (60 - 90 days) in the cytoplasmic projections and in the deeper parts of the non-ciliated cells. During late pregnancy there are only traces along the free border of the cells and in the cytoplasmic projections. A diffused positive PAS reaction for glycogen is also noticed in the secretion in the lumen of the tube. The small glycogen granules that appear in some of the tubal cells are located at the sides and basal

areas of the cells. These intracellular granules are not considered as secretory granules due to their inconsistency and their absence in the apical secretory part of the cells. They are possibly excess sugar stored as glycogen inside the cells.

PAS positive material, which continued to stain after diastase digestion, also with Alcian blue in varying degrees of intensity, and gave a B-metachromatic reaction (purple), is noticed at the distal border of the tubal cells and in the bulk of the cytoplasmic projections. During the first half of pregnancy, this material is confined to the cytoplasmic projections, then it gradually diminishes in the second half of gestation and reappears during late pregnancy about the 140th day till full term. This material which is similar to that found in the oviduct epithelium of non-pregnant sheep as a secretion is probably a glyco- or mucoprotein.

The isthmus of the oviduct does not show marked changes during pregnancy. There is a PAS positive reaction at the distal border of the lining epithelial cells. The reaction is similar to that in the ampullary region, i.e. diastase resistant, Alcian blue positive, and B-metachromatic. Though this material is mainly confined to the distal border of the cells it can be seen in the deeper part of a few cells.

Generally it can be stated that the tubal epithelium of pregnant sheep contains a carbohydrate-protein complex material mainly in the cytoplasmic projections and at the

distal border of the cells. Glycogen granules are found usually in the vicinity of the extruded cytoplasm and nuclei and in some of the epithelial cells. It probably appears as a result of excess sugar converted and stored as glycogen.

Acid and Alkaline Phosphatases - Traces of activity of Alkaline B-glycerophosphatase are noticed in the ampullary region of the oviducts of pregnant sheep. These are confined to the free border of the tubal cells, cytoplasmic projections and the lumen between the mucosal folds. The enzyme activity is confined to a few areas of the epithelium in the sections chemically fixed. Sections of freeze dried tissues show activity throughout the whole of the free border of the cells and especially in the cilia (Fig. 46). The freeze dried tissues referred to do not represent all the stages of gestation because improvement of the technique which has given the best results was only applied to the last batch of specimens collected. Comparison of the results, therefore, in succeeding stages of gestation would depend on presence or absence of any activity at all. However, according to the technique of freeze-drying employed for most of the tissues collected, the activity of the enzyme appears in the lumen of the oviduct between the mucosal folds and the neighbouring free border of the cells. During the first half of gestation the activity is confined to small areas in the lumen of the oviduct but during the second half of pregnancy the activity is more apparent at the free border of the cells

and even in the inner lining of some of the blood vessels in the subepithelial layer.

In the isthmus region of the oviduct the activity is more apparent than in the ampullary region and is constantly present throughout gestation at the distal parts of the tubal cells and in the lumen (Fig. 47). It is interesting to note that the enzyme activity is present at the same areas which show PAS positive reaction though the PAS reaction may extend deeper in the cells.

Acid B-glycerophosphatase activity could not be traced in chemically fixed tissues (neutral formalin) from the ampullary region of the oviduct. However, freeze dried tissues show activity along the distal border of the tubal cells, especially the cytoplasmic projections and the apical portion of the cells. The activity of the acid phosphatase appears much greater in this region of the oviduct than the alkaline phosphatase activity (Fig. 48).

In the isthmus region acid phosphatase activity could be demonstrated in chemically fixed tissues as well as in the freeze dried tissues. The enzyme has a similar distribution to that of alkaline B-glycerophosphatase but it is also present in the supranuclear part of the cells as well as in the distal border (Fig. 49). Acid B-glycerophosphatase activity is found to be reduced towards the latter part of gestation, starting in the ampullary region and proceeding towards the isthmus. It is present throughout gestation at the uterine end of the oviduct.

* The reduction in the activity of the enzyme is apparent even after the improvement of the freeze-drying technique.

Lipids - Lipid droplets are found in the tubal epithelium of pregnant sheep. Large lipid droplets are usually seen in the supranuclear part of the tubal cells, i.e. in the Golgi region. Lipid droplets of varying sizes are noticed in other parts of the cells, in the cytoplasmic projections and in the cellular debris within the lumen of the tube. Scattered droplets are encountered in the subepithelial connective tissue layer and in the muscularis. The lipid droplets are constantly seen in the apical part of the tubal epithelial cells throughout pregnancy (Fig.50). Using the acid-haematein technique as a parallel test for lipids, it confirmed the findings.

Ribonucleic Acid - The tubal epithelium of pregnant sheep shows traces of ribonucleic acid at the apical parts of the cells and in the cytoplasmic projections, especially early in gestation. As pregnancy advances, the ribonucleic acid staining weakens and gradually disappears from the cells and becomes confined to the free border of the cells and cytoplasmic projections. In the late part of gestation at about the 140th day of pregnancy, ribonucleic acid is found only in the cytoplasmic projections in some specimens. In other similar specimens it starts to reappear in the tubal cells.

In the isthmus the apical part of the tubal epithelial cells constantly shows the presence of ribonucleic acid and no marked changes are apparent during pregnancy. The staining of the ribonucleic acid varies in intensity and appears more intense during the early stages of pregnancy than during the rest of the gestation period.

Inorganic Iron - The presence of iron in the tubal epithelium of pregnant sheep is detected by the Perl's method. Prussian blue lake is found to be formed at the free border of the tubal epithelial cells, and on the cytoplasmic projections. Irregular deposits are seen inside some of the epithelial cells. A blue deposit is also noticed in the lumen of the tube and in the subepithelial connective tissue but the reaction appears diffused. The lining of the blood vessels also gives a positive reaction. This result is considered to show a minimum reaction for iron because most of the deposits are probably diffusion artifact. The pigment cells when present do not react to the Prussian blue test.

The Uterus and Placenta

Glycogen and Mucin - The distal border of the intact uterine epithelium of pregnant sheep is PAS positive diastase resistant. A similar substance is noticed in the walls of the blood vessels of both foetal and maternal tissues but more marked in the latter. This PAS positive material being resistant to diastase, B-metachromatic and Alcian blue positive, is considered to be a mucoid substance of a carbohydrate-protein complex (Pearse, 1960).

A PAS positive substance removable by diastase and stainable by Best's carmine (glycogen) is observed in the walls of blood vessels in the subepithelial connective tissue of the maternal septa (Fig. 51) and in the walls of the blood vessels in the Wharton's Jelly of the foetal villi. It is also found in some of the chorionic cells lining the

bases of the villi (Fig. 52). Glycogen in the maternal tissues only appears when the placentomes reach their full development but that in the walls of the foetal vessels appears in the early stages of pregnancy.

The binucleate cells in both the foetal and maternal lining show a PAS positive substance resistant to diastase in their cytoplasm. In the chemically fixed tissues the binucleate cells show the PAS substance in the infranuclear part of the cells, the nuclei appear eccentric and occupy the apical part of the cells towards the free border (Fig. 53). In the freeze-dried tissues this condition is not apparent and the PAS substance appears evenly distributed in the cell cytoplasm. Only when the nuclei of the binucleate cells occupy apical positions, the PAS substance is seen infranuclear (Fig. 54). The PAS substance is noticed also in the binucleate cells being detached from the chorionic epithelium and lying free in the space between the foetal and maternal tissues or just entering the lining of the maternal tissues. The binucleate cells lodged in the lining of the crypts tend to multiply and become multinucleated giant cells and at the same time their PAS substance becomes weaker and starts to disappear. Those giant syncytial cells lining the crypts are believed to have originated from the migrated binucleate cells and show little or no PAS substance in their cytoplasm.

However, this PAS substance which is confirmed by parallel tests of Alcian blue and B-metachromasia to be a carbohydrate-protein complex, appears to be a characteristic

property of the binucleate cells of the trophoblast and is never found in the uterine epithelial cells of the sheep except at their distal border. This clearly indicates that the binucleate and multinucleate giant cells lining the maternal crypts are most probably of trophoblastic origin.

The secretion in the lumen of the uterus, especially at the tapering end of the horns, gives a strong PAS positive reaction resistant to diastase (Fig. 41) and there is a similar reaction in the ducts of the glands. The secretory parts of the glands are always PAS negative though the glandular tissue appears active. The uterine glands, therefore, are believed to secrete the precursors of the PAS substance in the secretion found in the lumen of the uterus. However, the uterine glands show intense reactions for certain substances directly or indirectly taking part in the production and transfer of substances necessary for the nutrition and growth of the embryo. Amongst these substances are:-

1. Acid and Alkaline Phosphatases

The activity of Alkaline Phosphatase (B-glycero-phosphate substrate) is found in the uterus and placenta of pregnant sheep. It is found in the uterine glands and their lumina, the surface epithelium, the binucleate cells on the foetal and maternal sides, and in the endothelial lining of the blood vessels and capillaries of the endometrium, especially the maternal septa (Fig. 55). The activity of alkaline phosphatase, where present, is limited

to the periphery of the cells. The maternal tissues show more activity than the foetal tissues where it is almost entirely restricted to the binucleate cells of the trophoblast and the distal border of other chorionic cells (Fig. 56). The inner lining of the maternal blood vessels even in the deep parts of the stroma is distinguished by its intense reaction for alkaline phosphatase (Fig. 57). The syncytial lining of the maternal septa also shows intense activity. Tissues treated by chemical fixation (cold neutral formalin) and freeze-drying techniques both show the activity of alkaline phosphatase but in the chemically fixed tissues the reaction appears diffused when compared to that of the freeze-dried tissues.

As pregnancy advances, the activity is reduced, but it persists in the endothelial lining of the maternal blood vessels, the peripheral part of the binucleate cells and the lining syncytium of the maternal crypts. The columnar cells of the chorion show little or no activity, especially those at the bases of the villi.

In the intercaruncular areas the distal border of the uterine and glandular epithelia and the secretion in their lumina show constant enzyme activity throughout most of the gestation period. This enzyme activity persists in the inner lining of the blood vessels and in the round cells of the endometrium. The intraepithelial lymphocyte-like cells and those in the stroma show activity of the enzyme.

<u>The activity of Acid Phosphatase</u> is also noticed in the uterus and placenta. The intercaruncular areas show

enzyme activity in the surface epithelial cells and the uterine glands (Fig. 58). The reaction is present in the apical parts of the cells and at their border. The chorionic epithelium also shows enzyme activity in its binucleate cells and along the borders of the other cells. The secretion in the lumina of the glands and between the foetal and maternal tissues also shows enzyme activity (Fig. 59). In the caruncular areas the maternal tissue shows more acid phosphatase than the foetal tissues, especially in the walls of the maternal blood vessels and the lining of the maternal crypts. The reaction in the binucleate cells is more intracellular and diffused than that of alkaline phosphatase (Fig. 60). The presence of acid phosphatase activity in the lining of both foetal and maternal tissues indicates the importance of this enzyme in the transport mechanism of substances across the placental barrier.

The enzyme activity is noticed in the tissues throughout gestation though the intensity of the reaction is lessened during late pregnancy, especially in the cotyledonary areas. The red blood corpuscles show a weak positive reaction for acid phosphatase and the walls of the small vessels in the maternal septa are strongly positive even in late pregnancy.

2. Lipids - Lipid droplets are found in the uterine and glandular epithelia of gravid uteri of sheep as well as in the stromal tissues. They are concentrated at the supranuclear parts of the cells throughout gestation but many

droplets are noticed in different parts in the cells, at the sides and the infranuclear parts (Fig. 61). Numerous large lipid droplets are scattered in the endometrial stroma. At the tapering parts of the horns, lipid droplets are noticed in the endometrial tissues and in the debris within the uterine lumen.

The placentomes also show lipid material in both foetal and maternal sides but it appears more in the maternal side, especially in the lining cells (Fig. 62). The chorionic cells show basal lipid droplets of different sizes in the tall columnar cells lining the bases of the villi (Fig. 63) but they are less in the cells lining the sides of the villi. Binucleate cells contain lipid droplets around the nuclei. A few droplets are noticed even in the foetal connective tissue. Comparing the distribution of phosphatases and lipids, it is found that they are similar in that greater quantities are present in the maternal than in the foetal tissues. A parallel test for lipids was carried out using the acid-haematein method (Baker, 1946); it confirmed the results obtained by Sudan Black B method after the controlled chromation (Elftmann, 1958).

3. Ribonucleic Acid - In the intercotyledonary areas this is present in the uterine epithelium and uterine glands of pregnant sheep (Fig. 64). Certain cells in the endometrial stroma show variable intensity for ribonucleic acid staining; these include the large round cells with brownish-yellow cytoplasm and some scattered lymphocyte-like

cells. The endothelial lining of the small blood vessels also stains faintly.

In the chorionic epithelium both the tall columnar cells and the binucleate cells show the presence of ribonucleic acid but the intensity of staining is less than in the uterine glands.

In the caruncular areas both foetal and maternal tissues show the presence of ribonucleic acid, the most intensely stained cells being the binucleate. The inner lining of both foetal and maternal blood vessels shows a positive staining for ribonucleic acid. At the bases of the villi the chorionic cells are slightly stained but those at the sides and the tips of the villi show a greater intensity of staining due to the presence of numerous binucleate cells. The cells lining the crypts also contain ribonucleic acid (Fig. 65).

As pregnancy advances, there is a decrease in ribonucleic acid except in the binucleate cells and the uterine glands. The necrotic tips of the maternal septa also give a positive staining.

At the tapering part of the uterine horns, the surface and glandular epithelia show ribonucleic acid and the necrotic part of the blastocyst and the secretion in the lumen of the uterus give only a weak reaction for ribonucleic acid.

4. Inorganic Iron - The gravid uterus of sheep gives a positive reaction for iron (Prussian blue reaction) at the free border of the surface epithelium and that of the

glandular ducts. Some of the glandular cells give a positive result. The endometrial stroma shows a diffuse Prussian blue reaction but large globules giving an intense reaction are found here and there in the stroma. These appear to be large pigment cells. The inner lining of the blood vessels and the secretion in the uterine lumen are Prussian blue positive. Red blood corpuscles react faintly to Perl's reaction.

Iron is not demonstrable in the caruncular areas of the placenta except in the tall columnar cells of the trophoblast at the bases of the villi. This is possibly due to the remnants of red blood cells phagocytosed and absorbed by these cells (Fig. 66).

DISCUSSION

The ewe is a seasonally polyoestrous animal in which the number of oestrous cycles varies according to the breed and the environmental factors. The oestrous cycle of the ewe has been intensely studied. The duration of the cycle in the sheep is reported by Marshall (1956) to be 16 - 18 days and by Cole and Cupps (1959) as 14 - 19 days with a mean of 16.4 - 17.5 days.

The period of desire - oestrous - in the ewe begins before the rupture of the follicle according to many investigators. Marshall (1903) states - "Oestrous in domestic sheep very rapidly succeeds pro-oestrum, so much so that the period of desire sometimes seems to coincide with pro-oestrum." Quinlan and Mare (1931) observe that - "It is not possible to record the time of rupture in relation to the length of oestrous, but there appears to be little doubt that it occurs towards the end of the period of oestrous." Grant (1933) also describes early oestrous when the ovaries contain large follicles showing signs of eminent rupture and late oestrous or early metoestrum when the ovaries contain recently ruptured follicles with blood clots.

In the present study the use of the term oestrous period has been avoided in the description of the cyclical changes in the oviduct and uterus of sheep because the specimens were of unknown history. Nevertheless the writer describes certain changes which take place during or towards late pro-oestrum which may actually correspond to

oestrous or early oestrous, e.g. during this stage secretion in the ampulla is noticed within the apical parts of the tubal cells and projecting from the surface of the cells. Secretion is continued and shows marked increase in the succeeding stage of the cycle - metoestrum. Increase in the height of the tubal epithelium is noticed earlier during the growth of the Graafian follicles and reaches its maximum at metoestrum. The subepithelial connective tissues become swollen and vascular. These results are in accord with those of Casida and McKenzie (1931-33), McKenzie and Terril (1937) and Hadek (1955). During the luteal or dioestrous stage, secretion of the tubal cells is continued but towards the late part of this stage the nuclei of the cells appear in different positions pushing the remains of secretion towards the lumen of the tube. The surface of the tubal epithelium becomes irregular due to the cytoplasmic projections from the free border of the cells. Some nuclei are also noticed projecting into the lumen. The subepithelial layer is less swollen, less vascular and infiltrated with lymphocytes. This condition of the tubal epithelium is also described by Casida and McKenzie, and McKenzie and Terril, during late dioestrous and pro-oestrous. Hadek (1955) describes a similar picture during early oestrous and he claims that the nucleated cytoplasmic projections become detached and the affected cells become rod-like structures. However, there is little variation in the descriptions of the condition of the oviduct towards the end of the cycle but the authors agree that the oviducts are inactive during this stage.

It is noticed that the isthmus region of the oviduct is less active and has a thick circular muscle layer which is believed to help in the transport of ova towards the uterine horn by rhythmic contractions. Edgar and Asdell (1960) report a valve-like action of the utero-tubal junction of the ewe and explain that this action appears to be the result of oestrogen-induced oedema and flexure of the wall of the utero-tubal junction. Schilling (1962) claims that the transverse mucosal folds are more marked in the sheep than in the ox. He reports that the sub-peritoneal musculature of the ligamental apparatus forms a system of multiple fibres running in all directions and so arranged that rapid closure of the ovarian bursa is possible, but he denies that the connective tissue of the oviduct becomes oedematous during oestrous. No particular effort is made in this study to describe the topography of the mucosal folds of the oviduct but they generally appear longitudinal in transverse sections and in a few of the longitudinal sections examined. No part of the isthmus wall is observed to be oedematous during any stage of the cycle.

As far as the author has been able to ascertain, the morphology and fine structure of the tubal epithelium in pregnant sheep is investigated in the present study for the first time. There are no marked changes in the oviducts during pregnancy. They remain small in size when compared to the enormous increase in the size of the gravid uterus. The histological structure of the oviduct resembles to some

extent that seen during late dioestrous. During the first half of pregnancy the epithelium of the infundibulum and ampulla shows finger-like cytoplasmic projections and many extruded nuclei above the surface of the columnar cells. A few ciliated columnar cells are encountered between the non-ciliated cells and the latter appear quiescent. During the second half of gestation the cytoplasmic projections appear fewer in number until near full term when a new phase of secretory activity begins and the condition of the epithelium becomes similar to that seen at early pro-oestrum. This may be explained as follows: the activity of the oviduct has been arrested by the disappearance of the follicular hormone and persistence of the corpus luteum throughout most of gestation. When the corpus luteum begins to regress, another phase of activity begins.

The ultrastructure of the tubal epithelium has been investigated under the electron microscope. The oviduct has been collected from a ewe at mid-pregnancy. The tubal epithelium shows both ciliated and non-ciliated cells in alternating groups. The non-ciliated cells show cytoplasmic projections extruded partly or wholly from the surface of the cells, and may or may not contain extruded nuclei. They contain a few electron dense granules, vesicles and endoplasmic reticulum. The surface of some of the projecting bodies possess irregular protrusions similar to microvilli. The ciliated cells have true cilia which possess central and peripheral dark bands and have

basal corpuscles within the apical parts of the cells. The ciliated cells also have microvilli between the cilia; these are continuous with the cell membrane. The ciliated cells show small vesicles but electron dense granules are not apparent.

The electron microscope findings support the view that the ciliated cells are non-secretory and perhaps have an absorptive function. On the other hand, the non-ciliated cells appear secretory in character though not active during pregnancy. Bjorkman and Fredricsson (1960) reported similar observations in the bovine Fallopian tube during the oestrous cycle. They found that the irregular protrusions at the free surface of the non-ciliated cells were as a rule long and slender during the follicular phase but shorter and bulkier during the luteal phase. The condition of the non-ciliated cells in the oviduct of the pregnant sheep resembles to a great extent the described condition of these cells in the bovine oviduct during the luteal phase. Bjorkman and Bloom also came to the same conclusion that the ciliated cells are non-secretory and that they possess true cilia and microvilli.

The secretory products of the sheep's oviduct during the oestrous cycle, according to the present histochemical findings, consist mostly of mucoid substances which are PAS positive diastase resistant, stainable by Alcian blue and exhibiting B-metachromasia with toluidine blue. These substances are considered to be carbohydrate-protein complexes according to Pearse (1960). Lipid droplets are

constantly present in the supranuclear parts of the tubal epithelial cells and other droplets are also found scattered within the cells just prior to and after the secretory phase. A few glycogen granules are noticed during late dioestrum - pro-oestrum in the cytoplasmic projections of the non-ciliated cells and some of these granules are found within the cells; these are perhaps excess sugar stored as glycogen.

The activity of both acid and alkaline phosphatases (B-glycerophosphate substrate) is detected in the sheep's oviduct using freeze dried tissues and Gomori's techniques (modification for acid phosphatase - Wachstein et al. (1962)). The activity of alkaline phosphatase is limited to the distal border of the cells. On examination by phase contrast, the activity is apparent mainly in the cilia of the ciliated cells and in the lumen of the tube. These results agree with the findings of Bjorkman and Fredricsson (1960) in the bovine Fallopian tube but these authors suggest that the presence of alkaline phosphatase at these sites which include the microvilli might possibly be associated with a resorptive function of the ciliated cells. Hancox and Nicholas (1953) examined under phase contrast paraffin sections of the duodenum directly after incubation in the Gomori substrate bath used for demonstration of alkaline phosphatase. They found that the phase contrast method provides a more complete and quantitative representation of enzyme in the striated border of duodenal epithelial cells. The activity of acid phosphatase, on

the other hand, is found mostly in the apical parts of the non-ciliated cells and their cytoplasmic projections. The different sites of activity of these two enzymes indicate that they have different actions; the acid phosphatase seems to be more concerned with the formation of secretion while the alkaline phosphatase seems to control the passage of substances either entering the cells or more likely leaving them (see histochemical findings in the uterus). The increase in the activity of both enzymes during the secretory phase of the cycle supports the idea that they are directly concerned with secretion of the oviduct.

Ribonucleic acid is found in the tubal epithelial cells in pro-oestrum and possibly in oestrum but it becomes less concentrated and appears towards the distal parts of the cells and in the cytoplasmic projections during the rest of the cycle. It is possibly concerned with the building up of secretory granules because it appears before the secretory phase.

Inorganic iron is detected mainly at the free border of the cells and the inner lining of the blood vessels in the subepithelial connective tissue. The Prussian blue reaction for iron (Perl's method) in the oviduct appears diffused. This diffusion which is not uncommon in the connective tissue layers is possibly due in part to the sensitivity of the method and most likely due to iron which may simply be combined with certain acid groups of the protein as is, for instance, the colloidal iron in Hale's

method for acid mucopolysaccharides (Pearse, 1960). Therefore, the sites of iron in the oviduct are difficult to determine.

Hadek (1954-55) has reported similar histochemical findings on alkaline phosphatase activity, acid mucopolysaccharides, and ribonucleic acid in the oviduct of the ewe during the different phases of the oestrous cycle. He was unable to demonstrate lipids in the tubal epithelium and he considered that the secretory material is an acid mucopolysaccharide according to Pearse (1952). The methods used in the present study, however, clearly show the presence of lipid droplets in the Golgi region as well as in other parts of the tubal epithelial cells. The PAS positive secretory material also proved to be a carbohydrate-protein complex as has now been found by Pearse (1960) who states at the same time - "that the acid mucopolysaccharides, assumed by myself and others to be capable of giving a positive though weak reaction with PAS, in fact do not react at all."

During pregnancy the sheep's oviduct has shown minimal quantities of PAS positive substance resistant to diastase and ribonucleic acid. These substances are found mainly in the cytoplasmic projections. Glycogen granules which were noticed during the late luteal phase were also apparent during pregnancy and increased as pregnancy advanced; this last observation has given more support to the idea that excess unused sugar is possibly stored as glycogen. The presence of lipid droplets in the

Golgi zone is probably due to the presence of the Golgi apparatus which contain them. Lipid droplets in other parts of the cells and in the cytoplasmic projections do not show any significant alteration during gestation and therefore these droplets do not indicate the activity of the tubal epithelial cells.

The presence of acid and alkaline phosphatases in the tubal epithelium in similar positions to those described during the oestrous cycle is noted, but the reduction in acid phosphatase activity as pregnancy advances is also apparent. The presence of alkaline phosphatase activity in the lumen of the tube and at the free border of the cells may be due to the uterine secretions; the activity is more apparent in the isthmus than in the ampulla. The histochemical findings in the isthmus are more similar to those in the uterine epithelium than to those in the infundibulum and ampulla.

The chemical substances in the oviducts of sheep during pregnancy, though they are present, do not indicate any activity of the tubal cells except near full term when secretory granules reappear in some of the cells. This activity is believed to start when the action of the luteal hormone has decreased or stopped due to the regression of the corpus luteum of pregnancy.

The Non-gravid Uterus

The cyclical changes in the endometrium of non-pregnant sheep have been studied by many investigators - Marshall (1903), Casida and McKenzie (1931-33), Grant (1933),

Cole and Miller (1935), McKenzie and Terril (1937), and Hadek (1954-55). They all agree that a period of growth occurs in the sheep's uterus during oestrous and metoestrous, that it is marked by congestion of the blood vessels, oedema of the stroma and an increase in the height of the epithelium. They also agree that growth of the uterine glands is apparent at metoestrum and that the glands become coiled and reach their maximum activity at the mid-luteal stage. During the same stage the epithelial surface becomes folded. Leucocytes are found in the epithelium in early dioestrous but they appear in greater numbers at the beginning of regression of the corpus luteum (McKenzie and Terril).

The present observations largely agree with the findings of the above mentioned authors. The pro-oestrous phase, as noted before, may include oestrous, and therefore proliferation of tissues is noticed at this time. The most marked changes are apparent during metoestrum when the endometrium shows congestion of blood vessels, hypertrophy of the epithelial and stromal tissues and active secretion by the glands. An increase in lymphocytic infiltration towards the end of dioestrum is also noted.

The presence of melanoblasts in the subepithelial layer has been reported by Grant (1933). In the present investigation these cells are also found to contain true melanin granules. They occur only in the uteri of some sheep even of the same breed. The findings of Grant that melanoblasts occur in the uterus during foetal development

and that they are not found invariably in the uteri of sheep indicate that they have no physiological importance. However, apart from these melanoblasts, there are other round cells scattered in the endometrial stroma and their cytoplasm is characterised by a brown-yellowish colour in sections stained by haematoxylin and eosin, or even unstained. These round cells also possess other chemical properties, they show PAS substance diastase resistant, ribonucleic acid, lipoid material of homogenous character and slight enzyme activity. Hadek (1955) has described round pigment cells in the uterus of the ewe, which have more or less similar properties, and he considered them to be of connective tissue origin and to contain lipopigments or pigments of wear and tear which they acquired from the neighbouring tissue rich in melanocytes. The writer also agrees that they are probably macrophages and that they have acquired their pigments and other substances from the neighbouring tissues. Other leucocytes which are believed to be of the lymphocytic group are also encountered in the stroma; these will be referred to in the discussion of the changes in the gravid uterus.

Histochemistry of the Non-gravid Uterus

The uterine and glandular epithelium appears secretory at certain phases of the oestrous cycle and a histochemical investigation is carried out to determine what type of secretion is formed there. The surface epithelium of the uterus shows a PAS positive substance diastase resistant only seen at the distal border of the

cells. Similar material is seen in the lumen of the uterus and the ducts of the glands but never inside the cells. The glandular cells are PAS negative and this clearly indicates that the uterine and glandular epithelial cells do not secrete a carbohydrate-protein complex which is found in the oviducts and lumen of the uterus. Therefore, the uterine glands either form the precursors of this substance or, as Hadek (1954) suggested, the PAS substance might be traced back to the secretion in the oviducts. The writer disagrees with Hadek's suggestion because in the present study it has been observed that in the lumen of the gravid uterus the secretion is strongly PAS positive while the oviducts show little or no activity during pregnancy.

The constant evidence of the activity of alkaline and acid phosphatases at the distal border of the epithelial cells may indicate that these enzymes are concerned with the transformation of the epithelial and glandular secretions as they pass out of the cells. The possible relationship between the mucins and the phosphatase activity will be discussed with the histochemical changes of the gravid uterus and placenta.

Ribonucleic acid is found in the uterine epithelium and glands. During pro-oestrum the surface epithelium and the deeper parts of the glands show ribonucleic acid, at metoestrum the quantity of ribonucleic acid increases in both uterine epithelium and glands, and during dioestrum it gradually decreases especially in the deeper parts of the glands. Hadek (1954-55) also observed maximum quantities

of ribonucleic acid in the uterus of the ewe during the secretory phase of the cycle. The ribonucleic acid in the sheep's uterus is considered to be important for the synthesis of uterine secretion (see Histochemistry of the Gravid Uterus).

Lipid droplets are constantly present in the supranuclear parts of the uterine epithelial and glandular cells. They are found in other parts of the cells as large droplets during late pro-oestrum and metoestrum and they are also found in the lumen of the uterus and glands. These findings are more or less in agreement with Hadek's and most probably the lipid droplets are secreted by the epithelial cells as one of the constituents of the uterine milk. The constant supranuclear lipid droplets might be contained in the Golgi apparatus.

Inorganic iron is detected in the uterine glands and the distal border of the uterine epithelium. The Prussian blue reaction is stronger during the late pro-oestrous and metoestrous stages than in the other stages of the cycle. Hadek (1958) also reported the presence of iron in the uterine glands. He claims that the changes in amount of inorganic iron are influenced by folliculin. He observed that a few granules are occasionally found in the glandular lumen. However, in the present study, iron is also found in greater quantities during pregnancy and this indicates that it forms part of the uterine secretion and therefore it is more apparent during the activity of the uterine glands.

The Gravid Uterus and Placenta

The study of the placenta of sheep has received much attention due to its unique position between the deciduate and non-deciduate placentae and its importance in the classification and evolution of placentation. In this study the changes in the gravid uterus of the ewe and the development of its cotyledonary placenta, especially the foetal-maternal relationship which up to the present time is uncertain, have been further investigated.

The condition of the uterus before implantation was found by the author to be similar to that of early dioestrous. The endometrium appears vascular, oedematous and infiltrated with lymphocytes. The uterine and glandular epithelium is tall columnar and the glands are active. Assheton (1906) described an increase in the complexity of the glands and their secretion, a slight thickening of the stroma and an invasion of the epithelium by leucocytes as the only features which mark the 15-18 days of pregnancy. The present results also agree with the implantation phenomena related by Amoroso (1952).

Very early in pregnancy (5 mm. C.R.L. embryo) the endometrium is found denuded of epithelium at the sites of contact with the blastocyst. Lymphocytes have migrated towards the surface of the endometrium and around the glands. Aggregates of lymphocytes are seen in the lumina of the glands. Vascularisation and oedema of the endometrium are apparent. Before the end of the first month of pregnancy the endometrium is denuded of its lining

epithelium in most of the caruncular and intercaruncular areas. Moreover, in the caruncular areas, destruction of the endometrial stroma is noticed in the form of tracks at right angles to the surface of the caruncles. Pycnotic nuclei are predominant along these tracks. The chorionic epithelium facing these areas appears folded and contains among its tall columnar cells, spherical dark staining binucleate cells. The destruction of the uterine epithelium has been reported by Assheton (1906), Wimsatt (1950), and Amoroso (1951), who believed that it was brought about by the agency of the dark staining binucleate cells of the trophoblast which migrate through the epithelium and come to lie on the underlying stroma and form a syncytial lining of the maternal tissues. (The origin and nature of the cells lining the maternal crypts will be referred to later in the discussion.)

The Formation of the Crypts and Villi

In the present study individual dark staining binucleate cells are found on the surface of the naked endometrial tissue and on the sides of the ridges between the tracks of the degenerating tissue. Multinucleate cells of similar characters are also encountered at the same sites, especially at the bases of the developing crypts. As pregnancy advances, the degenerating tissues disappear leaving deep maternal crypts which are simultaneously occupied by the folds of the chorionic epithelium (primitive villi). Later in pregnancy the foetal villi grow deeper and they increase in length and

branch. The subsequent growth of the foetal villi is described by Wimsatt (1950). The crypts also develop, and intercrypt columns or septa of the maternal tissues are found in the intervillous spaces.

The formation of the foetal villi and maternal crypts has been described by Assheton (1906), Wimsatt (1950) and Amoroso (1952). They all agree that the foetal villi appear first as buds or prominences of chorionic cells into which cores of mesoderm and allantoic vessels follow; they then fit or penetrate into the crypts, increase in length and branch as pregnancy advances. The present results agree with their findings as far as the formation of the foetal villi is concerned. Amoroso (1952) states that - "Whether the villi literally grow into the maternal tissues either mechanically or by a phagocytic action is uncertain." In the present study it has been observed that the penetration of the foetal villi follows a destruction and disappearance of stromal tissues. This is believed to be due to an ingrowth of binucleate cells perhaps aided by other chorionic cells causing some process of digestion and absorption but not true phagocytic action. This is in agreement with Assheton who also states - "that the binucleate cells, by becoming firmly attached (actual protoplasmic continuity having been acquired), to certain small areas of the maternal stroma, the future fundus of the crypt, retard at those spots the increase of the rapidly swelling up trophospongia which is now taking place around it and so allow of the envelopment

of the villi by this expanding trophospongial tissue." The present findings also agree to a certain extent with those of Hamilton, Harrison and Young (1960) in the Fallow deer placenta, who state that - "In the Dama the crypts develop before the primitive villi are formed. The caruncular epithelium exhibits localized areas of hyperplasia, the central regions of which become necrotic. The epithelial invagination becomes canalized as a result of the disappearance of the necrotic material. The primitive crypts are arranged in rows transverse to the long axis of the caruncle (Strahl, 1906; Turner, 1879; Harrison and Hyett, 1954). The primitive villi enter the crypts after the disappearance of the central necrotic core and only after their establishment within the crypts are any binucleate cells seen within the crypt lining." Their belief that the lining of the maternal crypts is epithelial and maternal in origin is discussed later.

Degeneration of maternal tissue is also noticed at the tips of the maternal septa where the stromal tissue is found naked in many places facing the chorionic cells. This is most probably due to the phagocytic action of the chorionic cells at the bases of the villi; the tall columnar chorionic cells contain blood corpuscles, pigment granules and other cell debris.

<u>The Lining of the Maternal Crypts</u>

There has been much disagreement and uncertainty on the nature and origin of the lining of the maternal crypts in the placenta of ruminants. Amoroso (1952) on

syndesmochorial placenta quotes - "The nature and origin of the investing tissue of the maternal septa have proved to be exceedingly difficult and controversial ..... In the cow, Kolster (1902), Ledermann (1903), Jenkinson (1906), Pomayer (1919) and Chiodi (1927) believe them to be modified uterine epithelium. Hammond (1927), on the other hand, states that they are not true epithelial cells, 'but consist of connective tissue, plasma or lamella cells whose function is nutritional and which tend to congregate on the abraded surface.' ..... Nevertheless, it is quite clear, also, as is true of the placenta of the sheep and black-buck, that the cells lining the crypts are foetal, and trophoblastic in origin."

In this study, it is believed that the binucleate cells arise from certain primordia in the foetal ectoderm, migrate to the maternal side, and take part in the destruction of the endometrial tissues. As they migrate to the maternal side they increase in size and become closely connected to the underlying stroma or capillaries while some of them degenerate and disappear. Once they are attached to the maternal tissues they start to lose some of their chemical properties, e.g. they contain little or no PAS substance and ribonucleic acid. Their nuclei are believed to multiply within their cytoplasm thus giving rise to multinucleate cells which are found lining the bases of the crypts. The foetal origin of the binucleate cells has been detected by studying the early stages in the development of the placenta morphologically and histo-

chemically. The morphological study reveals that the binucleate cells and mitotic figures are first seen only in the chorionic epithelium; they later migrate to the maternal side of the placenta and the binucleate cells are noticed on the surface of the chorionic epithelium, in the space between the foetal and maternal tissues and on the surface or between the maternal stromal cells. The histochemical findings are in accord with Wimsatt's (1950) in that the PAS substance serves as a marker whereby the transformation of the cells could be followed. It is also found that the binucleate cells are rich in a variety of chemical materials in agreement with Wimsatt (1951) but the writer is unable to agree with him that the binucleate cells of the sheep are erythrophagocytic. The facts that the binucleate cells of the trophoblast are the first cells to invade the maternal tissues, that they are rich in a variety of chemical materials of known physiological importance, and that they are always nearest to the maternal source of nutrition throughout pregnancy, indicate their importance in the transport of materials to the trophoblast and thence to the foetus.

The electron microscopic findings in the junctional zone of the sheep's placenta show that the binucleate cells on the lining of the maternal tissues possess short microvilli when they are in direct contact with the trophoblast. The neighbouring syncytial and cuboidal cells possess tall microvilli which interdigitate with those of the chorionic cells. The binucleate cells rest on a basement membrane;

their plasma membrane in apposition to this basement membrane shows infoldings. The endothelial lining of the blood capillaries is also supported by a basement membrane which is only separated from that of the binucleate cells by an intervening space. The lining endothelial cells send projections into the lumen. These invaginations and processes may indicate the activity of the cells in the transfer of materials from the maternal blood stream to the trophoblast and vice versa. Similar findings were reported in the cow by Bjorkman and Bloom (1957) and in the Fallow deer by Hamilton, Harrison and Young (1960). Bjorkman and Bloom, comparing their findings with those found in swine placenta by Dempsey et al. (1955), concluded that the cryptal epithelium of the bovine placentome is of uterine origin and if the bovine placenta were of syndesmochorial type the microvilli would be expected between the cryptal epithelium and the endometrial stroma. Hamilton et al. found in the Fallow deer placentome very similar results to those described by the writer in the sheep's placentome. They conclude that - "The crypt lining in Dama consists of maternal cuboidal cells with apical microvilli, and in limited regions the lining is a syncytium also possessing microvilli. Binucleate cells, devoid of microvilli, are also included in the crypt lining." They believe that the syncytial lining is derived from the maternal cuboidal cells. However, they also state that - "if, on the other hand, they are derived from binucleate cells it means that the latter, after

invasion of the crypt lining, lose certain characteristic cytological features and take on those of the cuboidal cells of the lining proper. Neither Bjorkman and Bloom (1957) nor we have found any evidence for such a transformation."

Summing up, the present results are considered to show that the binucleate cells of the trophoblast migrate to the maternal side and become closely connected to the stromal tissues; they become enormously enlarged at the bases of the crypts and give rise to the multinucleate giant cells. During the growth of the placentome the multinucleate cells being closely connected to the growing stroma become stretched and flattened along the sides of the intercrypt septa and thus form a syncytial lining. The histochemical reactions show that the binucleate cells are rich in important chemical materials and on the maternal side they gradually lose some of these properties. The electron microscopic findings show that the binucleate cells are very near to the maternal capillaries only separated from them by a minute intervening space. When their free border is in direct contact with the chorionic epithelium they possess short microvilli; this possibly indicates that they are starting to acquire certain features of the maternal tissue. The syncytial lining of the crypts which is believed to be derived from the binucleate cells possesses apical microvilli, which interdigitate with those of the chorionic epithelium. The syncytial lining, therefore, is definitely involved in the

physiological exchange between the foetal and maternal sides. The infoldings of the basal plasma membranes of the binucleate cells may indicate "pinocytosis", a process by which particles can move from one side of a membrane to the other. The significance of membrane vesiculation has been discussed by Amoroso (1961) who considers that it has interesting features such as specificity of binding groups on the membrane which permit greater selectivity in the transfer operations and he surmised that membrane vesiculation might be a feature of certain ion-pump mechanism.

Blood Flow in the Placenta

Barcroft and Barron (1946) have described the form and relations of maternal and foetal vessels in the placenta of sheep. They have found that in the uterus the arteries extend to the mucosal surface and break up into capillaries and are drained by capillaries and veins parallel to them. In the foetal villi a central artery breaks up into a superficial capillary net which also drains into veins at the base of the villus. However, they have ascertained that the blood flows in parallel and opposite directions and that the foetal vessels and the supporting tissues reach maximum development before the maternal vessels. In agreement with Barcroft and Barron, the writer has observed that the maternal blood vessels run in a vertical direction towards the surface of the caruncle and that the foetal villi show central arteries which are parallel with their long axes. Both foetal

villi and maternal septa show small blood vessels and capillaries immediately under the lining cells. The vessels of the maternal septa give off lateral branches which are enveloped by the syncytial lining thus increasing the area of physiological exchange.

Towards the tips of the maternal septa some of the tissues appear necrotic and in some places extravasation of blood is observed between the maternal septa and the chorionic epithelium. The extravasated blood is engulfed by the tall columnar cells of the trophoblast and red blood corpuscles are observed within their cytoplasm. Extravasation of blood has been reported by Jenkinson (1906), Assheton (1906) and Wimsatt (1950). Assheton describes this condition as characteristic of the deciduate type of placenta. Wimsatt states that - "the escape of blood is sporadic and it is believed to be affected by the necrotic collapse of the terminal portions of the maternal septa induced by a static vascular congestion in the maternal capillaries." The writer's observations are in agreement with those of Wimsatt.

The histological structure shows hypertrophy of endothelial lining of the maternal vessels within the septa; this has not been observed in the lining of the foetal vessels. By special staining of the reticular tissue, it is found that all the blood vessels are covered by reticulum. Even at the tips of the maternal septa, where some of the vessels are not enveloped by epithelial cells, the reticular coat is there. This is in agreement

with Wimsatt (1950). In some places the reticular covering is comparatively thin and may be identical to the material contained in the intervening space seen under the electron microscope between the binucleate cells and the maternal capillaries. The writer prefers the use of the term "perivascular coat of argyrophil fibrils" to "coat of reticulum" because the silver impregnation technique stains other tissues besides reticulum. However, this clearly shows that neither the chorionic epithelium nor the syncytial lining of the maternal septa is in direct contact with the endothelium of the blood vessels.

In the light of the present observations and the findings of other authors, the foetal-maternal relationship in the placentome of the ewe is believed to be a syndesmochorial one. The morphological and histochemical evidences support this belief in many respects. The early appearance of the binucleate cells in the foetal ectoderm and their migration to the maternal side have been detected by the PAS technique at different stages of gestation and no evidence has been found that the binucleate cells are formed from the maternal tissues. There is also no sign of regeneration of the uterine epithelium within the placentomes but, on the other hand, degeneration of stromal and foetal tissues has been observed in all the stages of gestation. The presence of the multinucleate giant cells at the bases of the maternal crypts and their morpholigical and histochemical features make it evident that these cells have originated from the binucleate cells. The expansion

of the multinucleate cells along the sides of the crypts supports the hypothesis that these cells form the syncytial lining of the crypts. Accordingly, the placental barrier should consist of the endothelial lining of the maternal vessels with their perivascular coat of argyrophil fibrils, the syncytial lining which is formed by the binucleate cells of the trophoblast, the chorionic epithelium, the embryonic connective tissue and the endothelial lining of the foetal vessels.

Recent findings by the electron microscope in the placentomes of certain ruminants are considered to provide more definite evidence regarding the foetal-maternal relationship. Bjorkman and Bloom (1957) describe an epitheliochorial relationship in the placentome of the cow. They consider that the interdigitation of the microvilli is between the chorionic cells and the uterine epithelial cells. Hamilton,Harrison and Young (1960) also describe in the Fallow deer placentome an epitheliochorial relationship. They consider the limited regions of syncytial lining to be derived from maternal cuboidal cells, but also admit that the binucleate cells are incorporated in the crypt lining, and are convinced that these cells have migrated there from the trophoblast. They state that if the syncytial lining is derived from binucleate cells it means that the latter lose their characteristic features and take those of the uterine epithelium. Although their findings agree to a great extent with those of the present author, they were not convinced that the binucleate cells give rise to the

syncytial regions found in the crypt lining. However, in the present study it is considered that the binucleate cells become incorporated in the crypt lining, that they gradually lose their PAS positivity and that they possess short microvilli when within the crypt lining; all these observations strengthen the evidence in favour of their transformation into the syncytial lining of the crypts.

In the intercotyledonary areas the foetal-maternal relationship is different from that in the placentomes. Though the uterine epithelium is destroyed in many areas by a similar process to that in the caruncular areas, it is observed that the uterine epithelium is restored after the second month of pregnancy and the relationship has eventually become an epithelio-chorial one, very similar to that seen in the sow by Amoroso (1950). The chorionic epithelium in apposition to the openings of the uterine glands is thickened and invaginated, giving rise to areolae. Uterine milk and debris are found in the spaces between these areolae and the openings of the glands. Similar findings in the intercotyledonary areas have been recorded by Assheton (1906), Wimsatt (1950) and Amoroso (1952) but the last mentioned author reports that the denudation of the epithelium persists until midway through the fourth month of pregnancy, after which the lining appears to be restored. The present observations are also in agreement with Assheton, Amoroso and Wimsatt, in that the uterine glands increase in length and complexity, they are of wide diameter and are functional throughout pregnancy.

The histochemical findings show that a copious flow of nourishment is poured out by the glands to be taken up by the trophoblast.

Lymphocyte-like cells containing intracytoplasmic granules have been observed within the uterine and glandular epithelium of pregnant ewes. In agreement with Kellas (1961), the granules in these cells are eosinophilic and contain a variety of chemical substances (see histochemical findings). There is a similarity between these cells and the lymphocytes and especially the plasma cells which are known to contain eosinophilic Russell's bodies and are presumed to produce antibodies. This similarity indicates their importance in the defence mechanism of the maternal tissues against foreign invasion. They are only apparent in the intercotyledonary areas where the destruction of the maternal tissues is comparatively little and these cells may possibly take part in suppressing the invasion by the trophoblast. Amoroso (1952) has noted that the uterine epithelium always persists around the opening of the uterine glands and he presumes that the glandular secretion in preventing the close adherence of the trophoblast at these points, shields the glandular epithelium from the phagocytic action of the binucleate cells. The present author has also noticed that the uterine epithelium persists in similar areas and he considers that it is also possible that the presumed production of antibodies by the intraepithelial granular cells may combat the invasion by the cells of the trophoblast, especially if the invasion is

antigenic and not phagocytic action. The intraepithelial granular cells are also found during the late part of pregnancy in greater numbers and the possibility that they are important as a defence mechanism against infection after shedding of the placental tissues should not be ruled out. During the oestrous cycle the lymphocyte-like cells do not contain granules but they possess similar chemical substances to those found in these cells during gestation. It is therefore possible that these cells originate from lymphocytes of the endometrial stroma and during pregnancy they may be stimulated by the implantation of the blastocyst to form these granules and become plasma-like cells.

In this study, large round cells are frequently encountered in the endometrial stroma. They are found to contain pigments as well as other chemical substances like PAS positive material, phosphatases and ribonucleic acid. Similar cells have been recorded by Hadek (1955) and the author, in agreement with Hadek, considers that they are fixed macrophages of the endometrial connective tissues, that they acquire their pigment content from the neighbouring tissues and that they are different from the true melanoblasts described by Grant (1933). Maximow and Bloom (1961) record that - "Although it is widely believed that macrophages produce antibodies, there is considerable evidence that both macrophages and lymphoid cells are involved. In the so-called plasma cell theories, antibodies are supposed to be formed chiefly by lymphoid cells transitional between the fixed reticular cells and the

mature plasma cells." However, the comparison of the lymphocyte-like cells with other cells of similar morphology and function is desirable but further investigation is necessary before the comparison could be of value.

Histochemistry of the Gravid Uterus and Placenta

The histochemical findings in the uterus and placenta of sheep have been described in the observations. Glycogen granules are found in the maternal septa, mainly in the walls of the blood vessels, when the placentomes are fully developed. Glycogen is also noted in some of the tall columnar cells lining the bases of the foetal villi and in the walls of the foetal blood vessels. The glycogen granules were demonstrable by both Best's carmine method and PAS technique using diastase digestion as a control. Fahmy (1953) found no glycogen in the placenta of sheep apart from very faint and fine granules situated at the distal parts of the uterine epithelial cells. He quotes - "no glycogen is detectable chemically in the placenta of the sheep (Huggett, 1948)". Fahmy and Huggett (1954) reported that in the sheep, glycogen is absent in the cotyledons but present in the intercotyledonary zone. In the present study the writer has been unable to detect glycogen in the distal parts of the surface epithelium. These areas continue to show PAS positive reaction after diastase digestion and stain also by Alcian blue, the basement membrane of the surface epithelium showing a similar reaction.

The binucleate cells of the trophoblast contain a PAS substance resistant to diastase; this substance is

constantly present throughout pregnancy and by parallel tests it is found to be a carbohydrate-protein complex. The substance serves as a marker whereby the migration and transformation of these cells can be followed from the foetal to the maternal side as reported by Wimsatt (1950). When the binucleate cells become attached to the maternal side they increase in size, their nuclei multiply and they gradually lose the PAS substance in their cytoplasm.

The uterine secretions in the lumen are strongly PAS positive diastase resistant and these are found in great quantities in the tapering parts of the uterine horns. On the other hand, the cells of the uterine glands always give a PAS negative reaction though they are active and rich in a variety of other chemical substances. Therefore, a transformation of the secretory granules of the uterine epithelium and glands into PAS substances is suggested. This transformation may either take place when the secretion is leaving the cells or thereafter.

Alkaline phosphatase activity is found at the free border of the uterine and glandular epithelium, in the lumen of the uterus and glands, in the endothelial lining of the maternal blood vessels, in the binucleate cells of the trophoblast and in the lining of the maternal crypts. The peripheral localisation of the enzyme activity is common in all these sites and the activity is greater in the maternal septa than in the chorionic villi. These results are in accord with those of Fahmy (1953). In the present study a possible relationship between alkaline phosphatase

and the carbohydrate-protein complex in the lumen of the uterus and glands is suggested. Wislocki and Dempsey (1945) suggest a possible relationship between alkaline phosphatase and glycogen and state that - "phosphatases have been identified in nearly all animal tissues, and are now known to catalize a large number of important chemical reactions. Phosphatases are concerned with carbohydrate metabolism, phospholipid metabolism and calcium deposition." They add that - "the enzyme should be demonstrable in a region supervening between the source of sugar and the location where glycogen deposition is occurring." The present results agree with their statement that the transmission of sugar, possibly combined with other substances, from maternal to foetal tissues involves its passage across regions in which phosphatase is demonstrable. The presence of alkaline phosphatase in the endothelial lining of the blood vessels, i.e. between the source of sugar, the maternal blood, and the glycogen in the tunica media of the blood vessels supports the suggestion of Wislocki and Dempsey. Alkaline phosphatase is present at the distal border of the uterine and glandular epithelial cells, i.e. between the PAS negative substances within the glandular cells and the PAS positive substance just outside these cells, and this also supports the suggestion made by the writer. Huggett (1961) on carbohydrate metabolism in placenta and foetus concludes that - "The carbohydrates therefore appear to be needed as reserves for the synthesis of some particular component only required in

microquantities. It would seem that probably all mammals partake of both these carbohydrates (Fructose and Glycogen) in their foetal membranes and foetal fluids. But with the Ungulata and whales the glycogen is minimal, and does not appear to increase in hyper-glycaemia, whereas fructose does." This report shows that carbohydrates are possibly required for synthesis of other substances and the blood sugar is converted into fructose in the foetus. However, the transformation and transfusion of substances from the maternal blood to the foetus appear to involve many chemical processes which need enzymes to catalize their reactions; these are beyond the scope of this investigation.

Acid phosphatase is demonstrable in the uterus and placenta of sheep in sites similar to those of alkaline phosphatase but the activity appears more intracellular than that of alkaline phosphatase, i.e. in the apical parts of the cells. Fahmy (1953) gives a more or less similar description but he stresses the presence of the activity in the nuclei. The staining of the nuclei is considered in the present study to be a diffusion artifact because most of the tissues in a section give the staining reaction for acid phosphatase after long incubation periods. The freeze-dried sections flattened and fixed by absolute alcohol are found to give the best results for acid phosphatase. Gomori (1956) pointed out that certain artifacts such as nuclear staining were found impossible to avoid. He also states that - "The results obtained by the lead

sulphide and by the post-coupling method of Rutenburg and Seligman are closely similar. However, there are minor differences which appear to be due partly to the presence of more than one acid phosphatase in the tissues, partly to diffusion artifacts." Deane and Dempsey (1945) using the freeze-drying technique found a well defined concentration of acid phosphatase in the Golgi region of the uterine epithelium of pregnant cat and sow. They consider that the cells are engaged in active secretion of the enzymes since phosphatase is found in the lumen of the uterus. They also state that - "Nevertheless, we should emphasize that acid phosphatase is not a usual constituent of the uterine epithelium, for we have never observed it in the uterine epithelium from mon-pregnant individuals of any species." They add that - "The function of acid phosphatase is even more obscure than is that of alkaline phosphatase. Histologically its augmentation can be associated with increased ribonucleoprotein (RNA) metabolism (Bodian and Mellors, 1945)." It should be noted that in the present study acid phosphatase has been demonstrated in the uterus of non-pregnant sheep and its association with the uterine secretions have been discussed.

Ribonucleic acid unlike the carbohydrate-protein complex substance is found within the uterine and glandular epithelial cells as well as in other parts of the placenta. It is observed in the chorionic epithelium, the inner lining of the blood vessels and the syncytial lining of the crypts, but it is more intense in the binucleate cells and

the uterine glands. A faint staining of ribonucleic acid is observed in the secretion in the uterine lumen and the necrotic parts of the blastocyst. The round cells in the endometrial stroma and the lymphocyte-like granular cells contain ribonucleic acid. In the present study it is considered that the ribonucleic acid possibly takes part in the synthesis of secretory granules and thereafter forms part of the uterine secretions. Wislocki and Dempsey (1945) found cytoplasmic basophilia (ribonucleoprotein) confined almost entirely to the glandular and surface epithelium and showed considerable variation from species to species, at different stages of the cycle and during pregnancy. Wislocki and Dempsey (1946), comparing the pig's placenta with placentae of other mammals, observed that in man, cat and rodents the basophilia is intense in the first half of gestation but gradually disappears whereas alkaline phosphatase appears and gradually increases during the last half of pregnancy. In the sow they observed intense and rather long lasting basophilia of the trophoblast, but found no alkaline phosphatase in the trophoblast at any period (excepting the brush border of the columnar cells of the chorionic fossae). The writer has not observed this inverse relationship between ribonucleic acid and alkaline phosphatase in the present study. There is a direct relationship in the sheep's placenta between the two substances; this is especially clear in the uterine glands and the binucleate cells of the trophoblast which continue to show both the

enzyme and ribonucleic acid throughout most of the gestation period. However, Wislocki and Dempsey also suggest that alkaline phosphatase causes hydrolysis of ribonucleoproteins and they regard the ribonucleoproteins as being proteolytic enzymes engaged in protein and hormone synthesis.

Lipid droplets are observed in the uterine and glandular epithelium, the endometrial stroma, and the placentomes on both foetal and maternal sides. There are more lipids in the maternal tissue than in the foetal tissue. In the tall columnar cells lining the bases of the foetal villi, lipids are found towards the base of the cells; also the binucleate cells at the sides and tips of the villi show lipid droplets in their cytoplasm. The lining of the maternal crypts contains greater quantities of lipids, and the uterine epithelium and glands constantly show lipid droplets throughout most of gestation. It is noted that there is an association between the distribution of lipids and that of the phosphatases in the sheep's placenta and therefore it is most probable that the phosphatases also take part in the metabolism of phospholipids as has been reported by Wislocki and Dempsey. In the intercotyledonary areas there is no doubt that lipids constitute part of the uterine secretions since they are found in the uterine milk. Jenkinson (1906) has reported that fat globules are secreted by the uterine glands as an apocrine secretion and are quickly absorbed by the trophoblast. The presence of lipid droplets in the Golgi zone

of the epithelial cells has been discussed in the histochemistry of the oviducts and non-gravid uterus. Wimsatt (1951) has demonstrated two classes of lipids in the binucleate cells of the sheep; the first class of lipids in the Golgi region contains phospholipid, and the second class is present only sporadically in the giant cells and is of a grosser type. He considers the first class to represent the mitochondria of the giant cell and he also states that - "These observations suggest that the accumulation of fat in the giant cells is supervenient. It appears unlikely, therefore, that the giant cells play any significant part in the lipid metabolism of the placenta." Huggett and Hammond (1952) on the metabolism and transfusion of lipids report that - "In the syndesmochorial placenta of the cow and sheep, fat is histologically demonstrable in the uterine milk, and in the cells of the decidua, connective tissue and epithelium adjacent to the trophoblast cells, but only those which cover eroding villi." In the present study lipid droplets have been demonstrated in the chorionic epithelium and the binucleate cells of the trophoblast; it is uncertain whether these lipids are transmitted from the maternal to the foetal tissues almost unaltered or possibly as metabolic fat, produced in the placenta during intracellular metabolism.

Inorganic iron is demonstrable by the Prussian Blue reaction (Perl's method) in the uterine glands of pregnant sheep, at the free border of the surface and glandular

epithelium and in the lumen of the uterus. In the endometrial stroma there is a diffuse Prussian Blue reaction but a few large round cells are strongly positive, these cells being found to contain melanin pigments when examined in serial sections of the same blocks after staining by haematoxylin and eosin. Iron reactions are also observed in the inner lining of the blood vessels and even the red blood corpuscles are slightly stained. In the placentomes the tall columnar cells lining the bases of the villi give a positive Prussian Blue reaction and this is possibly due to the remnants of the red blood corpuscles being engulfed by these cells. This survey of the uterus and placenta of sheep for positive iron reactions clearly indicates that iron is made available to the embryo by two routes, the uterine secretions and the red blood corpuscles of the extravasated blood near the phagocytic cells of the trophoblast. Jenkinson (1906) also mentions the extravasation and ingestion of red blood corpuscles mainly in cotyledons; he describes that the corpuscles ingested by the trophoblast further aggregate and become paler and they may include yellowish brown pigment, or both corpuscles and pigment may be seen in one and the same cell and he did not get an iron reaction with these masses. Assheton (1906) has found pigments at the bases of the villi which according to his view may be regarded as an excretory product; he has been able to detect free iron in a few places in the chorion, and more rarely in the villi. Huggett and Hammond (1952) report that - "In the syndesmochorial

placenta of ruminants (with the exception of the cow) there are few haemorrhages from the maternal blood vessels at the roots of the villi. The blood pigments become absorbed by leucocytes which appear beneath the uterine epithelium, some of which contain unaltered hammoglobin, others iron-containing derivatives (Assheton, 1906)." Hoskins and Hansard (1964) on placental transfer and foetal tissue iron utilisation in sheep have found that there is more iron storage in maternal than in foetal tissues. However, though it is clear that the red blood corpuscles of the extravasated blood in the latter half of pregnancy make iron available to the foetus, it is considered that the uterine glands are the main source of iron for the growing embryo in the ewe.

The histochemical findings in the uterus and placenta of sheep indicate that the uterine epithelium and glands which are active throughout gestation are an important source of nutrients to the foetus. The uterine milk which is believed to be the product of the uterine secretions and cell debris contains carbohydrate-protein complex substances, acid and alkaline phosphatases, lipids, ribo-nucleoproteins or their derivatives, inorganic iron and damaged autolysing cells. All these substances or their precursors are found within the cells of the uterus and glands. Phosphatases and lipids are also found in the cotyledons and are observed to be in greater quantities in the maternal tissues than in the foetal tissues. They are most probably synthesised at these sites and play an

important part in the intracellular metabolism. The cotyledons are therefore considered as sites of physiological exchange, i.e. transfusion of certain substances to the foetus and excretion of waste products. Assheton (1906) states that - "the fact of accumulation of pigment in the bases of the villi leads one to suspect that the cotyledonary area is more concerned with excretion and possibly with respiration than with nutrition." Wimsatt (1951), discussing the significance of the binucleate giant cells, states that the binucleate cells without doubt have other specialised functions which they do not share with the columnar cells and in which those chemical substances, such as phosphatase and carbohydrate protein complexes which they exclusively contain, play a significant part.

In the present study, the binucleate cells of the trophoblast which are believed to migrate to the maternal side and give rise to the lining of the crypts are rich in phosphatases even after their transformation into the maternal syncytial lining. They are considered, therefore, to play a significant part in the physiological and chemical reactions which are necessary for the transfusion of material from the maternal sources to the foetus and vice versa.

GENERAL SUMMARY AND CONCLUSIONS

As has been mentioned in the Introduction, the literature on the reproductive tract of sheep is voluminous. The oestrous cycle and the cyclic changes in the reproductive tract of the ewe have been studied by many investigators for different purposes. The study of the placenta of sheep also received much attention due to the unique position of the cotyledonary placenta in the classification and evolution of the mammalian placentae.

In this study the morphological and histochemical changes in the oviducts, uterus and placenta of sheep, during the oestrous cycle and pregnancy, have been further investigated and new histological and histochemical observations have been added. Use of the electron microscope, freeze-drying and freeze-substitution techniques were of great advantage in the present study.

The material was taken from thirty-nine non-pregnant and fifty-four pregnant sheep of Scottish breeds; fourteen specimens of pregnant animals were of known history and the rest, of unknown history, were collected from the Edinburgh abattoir.

The changes in the oviducts during the oestrous cycle have been described in the text and have more or less confirmed the findings of previous authors. The tubal epithelium was found to be in a phase of growth during pro-oestrum and by the onset of oestrous to be in a phase of secretory activity, which gradually diminished towards the end of the cycle.

There were no marked changes in the oviducts during pregnancy and the author could find no reference to the morphology and the fine structure of the oviducts of pregnant sheep. In the present study, the fine structure and changes in the tubal epithelium of the pregnant ewe have been described; the epithelium was found to be quiescent and many cells were degenerating during the greater part of the pregnancy period. Only towards the end of gestation did any activity appear in the epithelial cells. The ultrastructure of the tubal epithelium revealed that it consisted of ciliated and non-ciliated columnar cells in alternating groups. It also showed that the ciliated cells are non-secretory and possibly have an absorptive function since they possess microvilli.

The histochemical investigation showed that the active tubal epithelial cells are rich in carbohydrate-protein complex substances. They also contain ribonucleic acid, lipids and acid phosphatase. Alkaline phosphatase was found only at the free borders of the cells and iron was also demonstrable in traces along the cell borders. The cyclical variation in the quantity and distribution of these substances within the cells has been described in the text and the secretions of the oviducts were considered to be mainly carbohydrate-protein complexes, i.e. glyco-proteins or mucoproteins. The significance of the other chemical substances found in the tubal cells and the lumen of the oviduct was discussed.

The uterine changes during the oestrous cycle were described. It has been observed that the cyclical changes

in the uterus follow closely those of the oviducts. The endometrium showed a period of growth preceded by vascularisation during pro-oestrum, a period of secretory activity during metoestrum and early dioestrum, and, finally, a period of regression and infiltration of leucocytes towards the end of the cycle. The present observations have largely agreed with the findings of previous authors. In the endometrial stroma melanocytes were encountered in some of the specimens collected and were considered to be of no physiological value. Other lymphocyte-like cells containing important chemical substances were encountered in the subepithelial layer and around the uterine glands; these have been discussed in relation to similar cells in the gravid uterus.

The uterine and glandular secretions have been studied histochemically and found to be rich in a variety of important chemical substances. The cells of the uterine and glandular epithelium showed the presence of ribonucleic acid, lipids, inorganic iron and acid phosphatase; alkaline phosphatase is active only at the free borders of the cells. The histochemical reaction at the free border of the uterine epithelium and in the lumen of the uterus and the ducts of the glands showed the presence of a carbohydrate-protein complex substance. Accordingly, a possible relationship between the phosphatases present at the free borders of the cells and the mucoid substance found outside the cells was suggested and discussed.

The study of the morphology and histochemistry of the gravid uterus and placenta of the ewe was a major part of

the present investigation. The changes in the gravid uterus and the development of the placenta have been described in the text at different stages of pregnancy. The condition of the uterus before implantation of the blastocyst was found to be similar to that of early dioestrous, i.e. the endometrium was in the active phase. When the blastocyst came in contact with the endometrium, the uterine epithelium was destroyed at the sites of contact and before the end of the first month of gestation the blastocyst had increased enormously and the endometrium was denuded of its lining epithelium in most of the caruncular and intercaruncular areas.

The destruction of the uterine epithelium and some of the endometrial stroma of the caruncles was considered to be due mainly to an ingrowth of dark staining binucleate cells of the trophoblast which migrated to the maternal side and possibly were aided by other chorionic cells. This destruction was believed to be caused by some process of digestion and absorption but not by true phagocytic action because the dark staining binucleate cells frequently seen nearest to or within the degenerating maternal tissues were apparently non-phagocytic.

In the present study the formation of the foetal villi and the maternal crypts in the sheep's placentome have been described. It was found that the crypts developed before the primitive villi were formed. The histological evidence showed that the maternal tissues had degenerated in the form of tracks vertical to the surface

of the caruncle, the degenerative tissues eventually disappeared and then folds of chorionic epithelium or primitive villi entered the evacuated crypts.

The fine structure of the junctional zone of a fully differentiated placentome was examined under the electron microscope and it was found that the foetal and maternal lining cells possess apical microvilli which were interdigitating with each other except where debris and secretory granules had intervened. Intracytoplasmic inclusions were found in both foetal and maternal cells; the former showed characteristic large vesicles near the microvilli while the latter showed characteristic electron dense granules.

The binucleate cells were rich in the electron dense granules and endoplasmic reticulum. When they were incorporated in the maternal lining, they possessed short microvilli interdigitating with the microvilli of the foetal cells. The basement membrane of the binucleate cells was separated from that of the endothelial lining of the maternal capillaries by an intervening space and their basal plasma membranes showed infoldings which could be an evidence of pinocytosis. The histochemical findings showed that the binucleate cells were rich in a variety of chemical substances, such as carbohydrate-protein complexes, ribonucleoproteins and alkaline and acid phosphatases. The carbohydrate-protein complex acted as a marker by which the migration of the binucleate cells was followed. Thereafter their transformation into multi-

The histochemical reactions showed that glycogen granules were present in the walls of the foetal and maternal blood vessels. The columnar chorionic cells at the bases of the villi contained glycogen. They contained inorganic iron which is believed to be liberated by the phagocytosed red blood corpuscles. Lipid droplets were also found towards the bases of these cells. Acid and alkaline phosphatases, lipids and ribonucleoproteins were observed in greater quantities in the syncytial lining of the maternal crypts than in the chorionic epithelium.

In the light of the present observations and the findings of other authors, the syncytial lining of the crypts being of foetal origin, the foetal-maternal relationship in the placentome of the ewe is believed to be a syndesmochorial one. The phosphatases and lipids in the binucleate cells and the syncytial lining were considered to be of vital importance in the transfusion of substances across the placental membrane.

In the intercotyledonary areas of the sheep's placenta, the uterine epithelium was eroded in the places which were in contact with the blastocyst during early pregnancy excepting that around the glands and at the junctional zone between the caruncular and intercaruncular areas and the tapering parts of the horns. The epithelium was believed to be destroyed by a similar process to that described in the caruncular areas. However, the uterine epithelium was restored in the intercotyledonary areas after the second month of pregnancy, and the foetal-

maternal relationship had eventually become an epithelio-chorial one. The chorionic epithelium in apposition to the openings of the uterine glands was thickened and invaginated giving rise to depressions known in the pig's placenta as "areolae". The uterine milk and debris were found in the spaces between the areolae and the openings of the glands.

The uterine glands increased in length and complexity and they were of wide diameter and functional throughout pregnancy. The histochemical reactions showed that a copious flow of secretion was poured out by the glands. The epithelial and glandular cells contained ribonucleic acid, acid and alkaline phosphatase, lipids, and inorganic iron.

Lymphocyte-like cells containing intracytoplasmic granules were observed within the uterine and glandular epithelium of pregnant ewes. The granules were eosinophilic PAS positive, stainable by pyronim Y, i.e. containing ribonucleic acid, and showed alkaline phosphatase activity. The similarity and relationship between these cells, the lymphocytes of the endometrial stroma and the plasma cells were discussed.

Large round cells containing pigments, PAS positive material, phosphatases and ribonucleic acid, were observed in the endometrial stroma of pregnant ewes and these cells were considered to be macrophages of the endometrial connective tissue.

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LIST OF ABBREVIATIONS

AAF	-	Alcohol acetic acid formalin - Lillie's fixative.
Bi	-	Binucleate cell.
CE	-	Chorionic epithelium.
Cy P	-	Cytoplasmic projection.
D	-	Debris.
E Gr	-	Eosinophilic granules.
En	-	Endothelium.
ER	-	Endoplasmic reticulum.
Ex B	-	Extravasated blood.
F	-	Foetal side.
H. & E.	-	Haematoxylin and eosin.
Inf	-	Infolding of basal plasma membrane.
M	-	Maternal side.
M Gr	-	Melanin granules.
MN	-	Multinucleate cell.
MS	-	Maternal septum.
MV	-	Microvilli.
N	-	Nucleus.
PAS & H.	-	Periodic acid Schiff technique and counter-stained by haematoxylin.
Syn	-	Syncytial lining of maternal crypt.
UE	-	Uterine epithelium.
U Gl	-	Uterine gland.
UL	-	Uterine lumen.
Vi	-	Foetal villus.

FIG. 1

Oviduct isthmus - pro-oestrum.

Tall columnar epithelium and thick circular muscle layer.

H. & E. X 500.

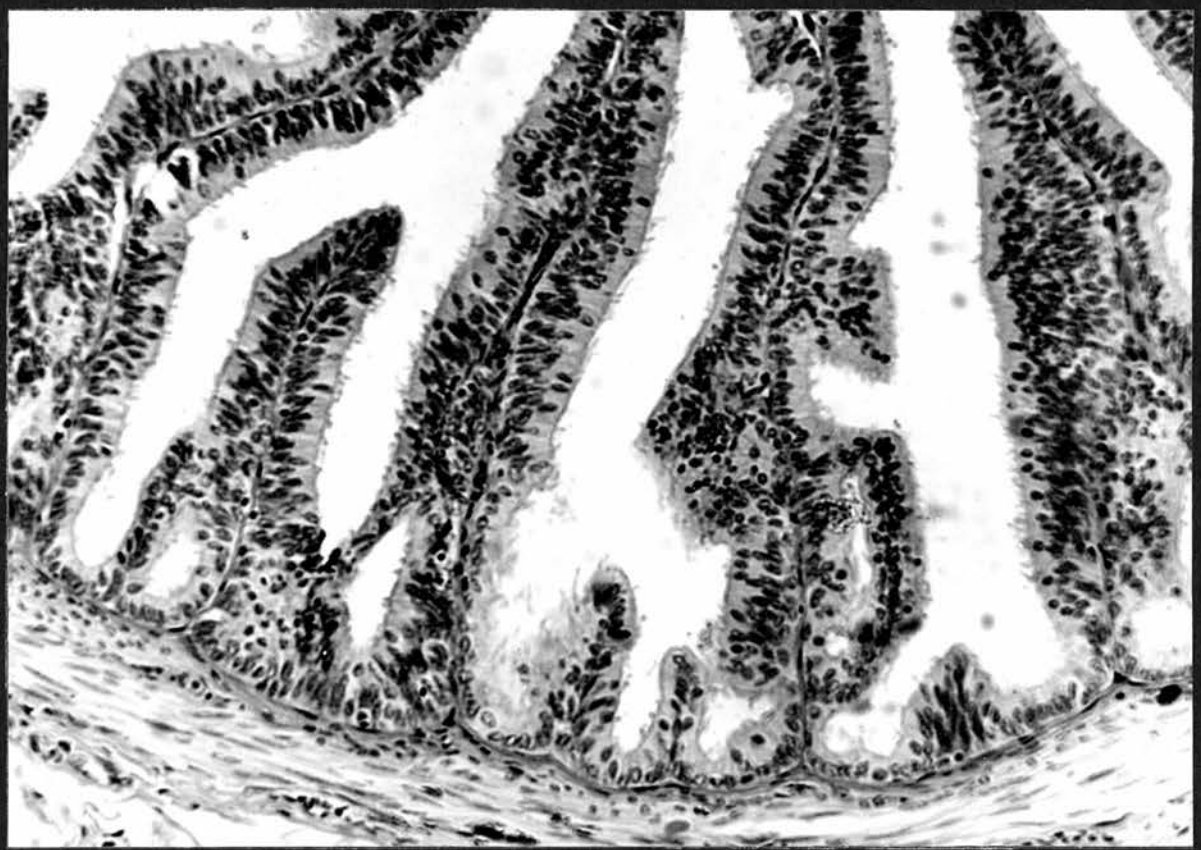


FIG. 3

Oviduct ampulla - pro-oestrum.

Tall tubal epithelium which tends to be pseudo-stratified. PAS positive (diastase resistant) secretion granules project from its free surface. Subepithelial layer is hypertrophied and vascular.

PAS & H. X 500.

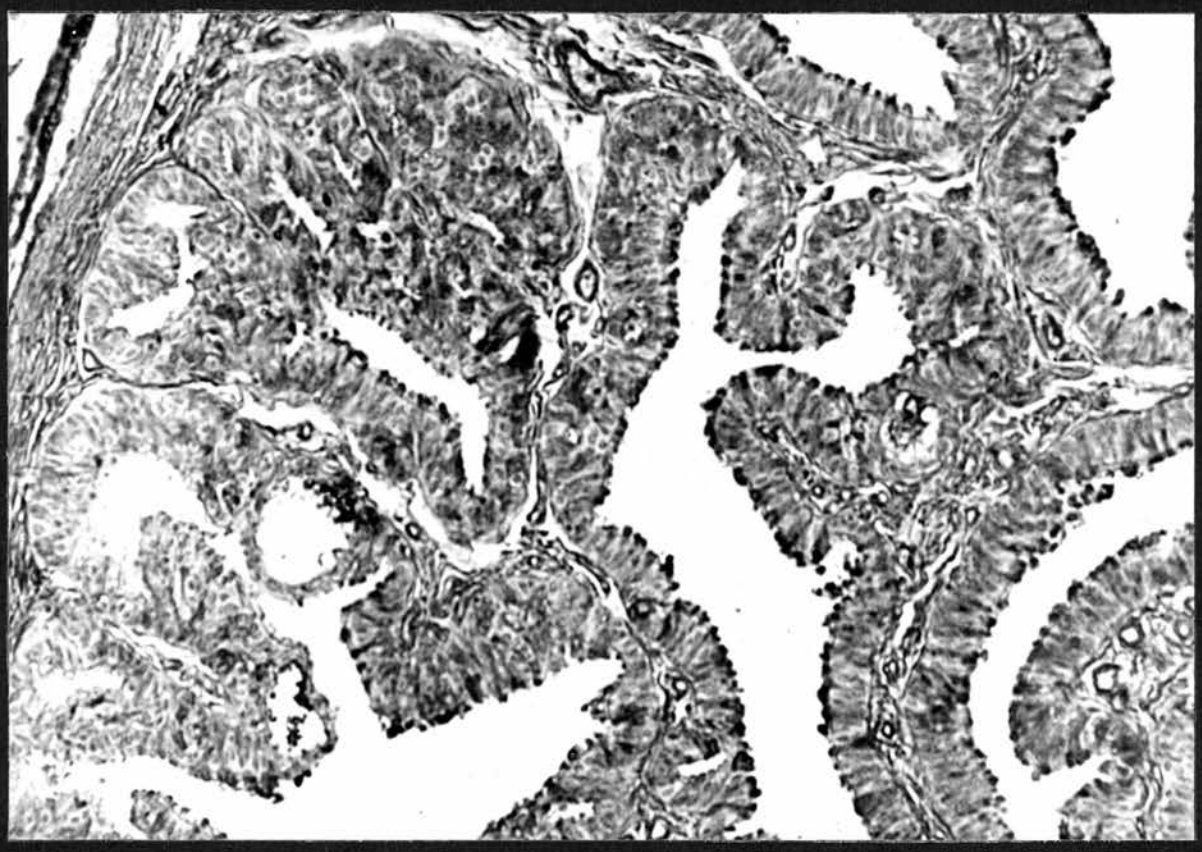


FIG. 4

Oviduct ampulla - metoestrum.

Pseudostratified tubal epithelium in a secretory phase. Mucosal folds are swollen and send short lateral branches.

H. & E. X 500.

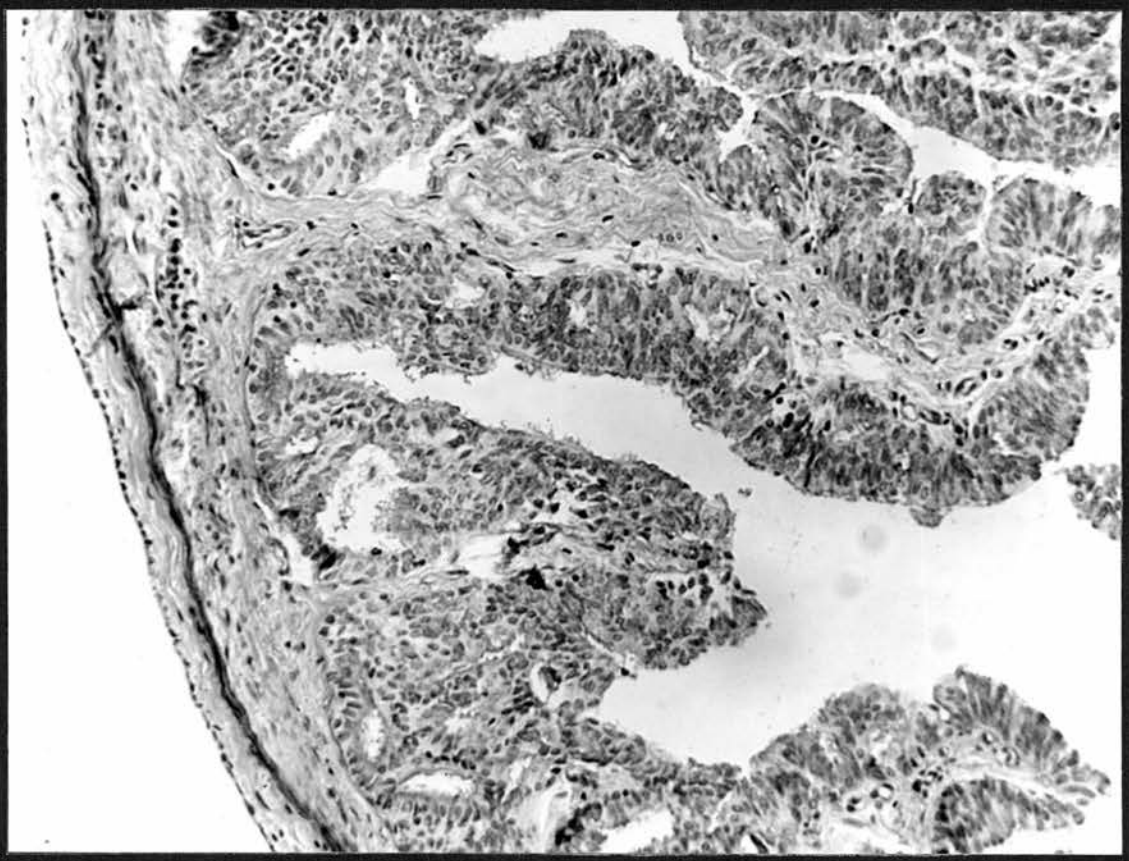


FIG. 5

Oviduct ampulla - late dioestrum.

Tubal epithelium shows irregular surface due to cytoplasmic projections; a few nuclei migrate to the surface. The subepithelial layer is less vascular and infiltrated with lymphocytes.

PAS & H. X 500.

FIG. 6

Uterus - anoestrum.

The uterine and glandular epithelium is simple columnar and shows no sign of activity. Infiltration of lymphocytes around glands and beneath surface epithelium.

H. & E. X 500.

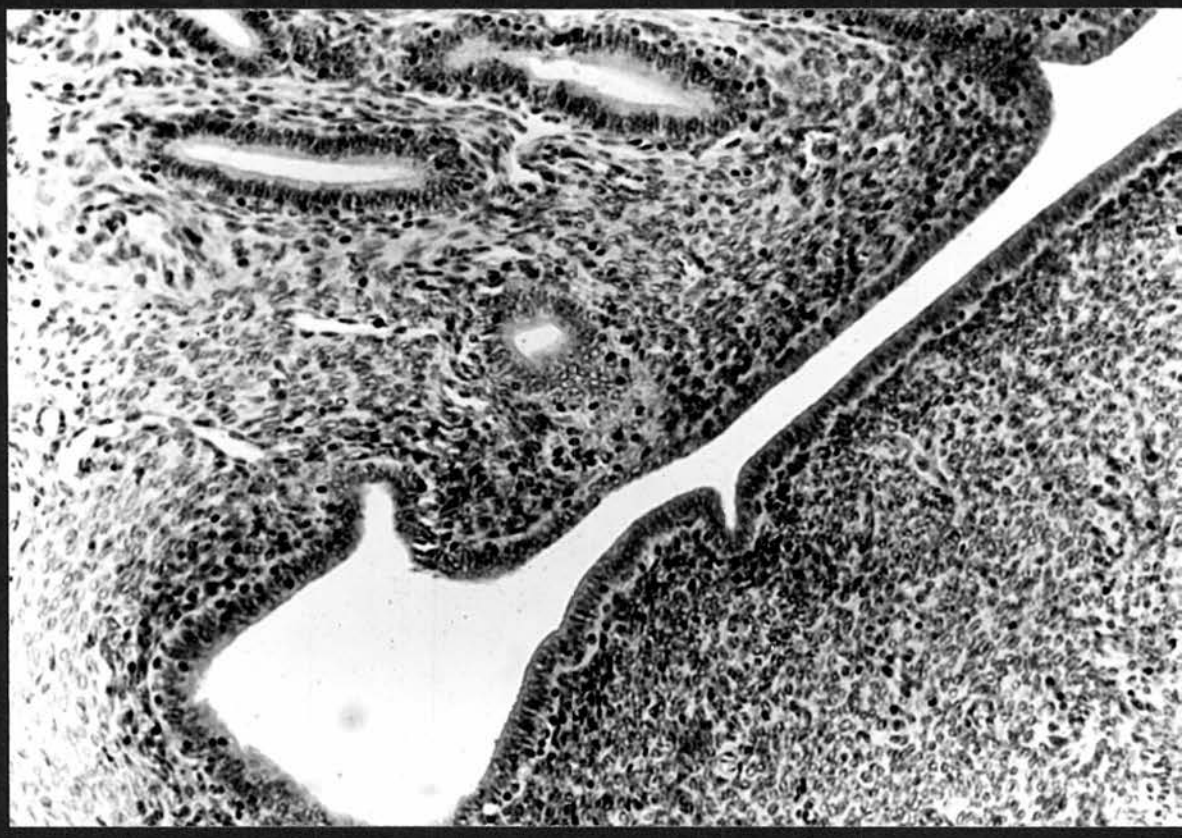


FIG. 7

Uterus - pro-oestrum.

Increase in the height of uterine and glandular epithelium. The stromal cells are hypertrophied and many vessels are apparent.

H. & E. X 500.

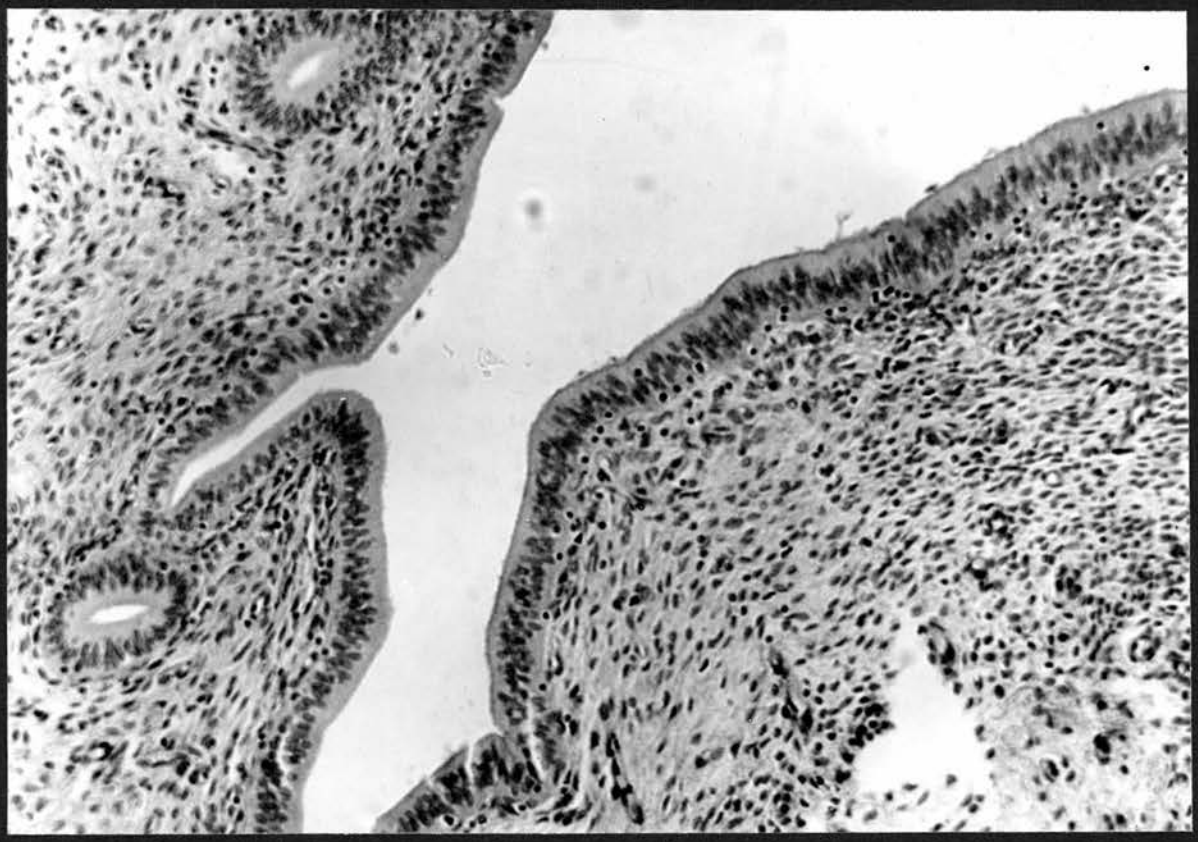


FIG. 8

Uterus - metoestrum.

The uterine epithelium is tall and hypertrophied. The endometrial stroma is loose and its blood vessels are congested. Note lymphocytic infiltration.

H. & E. X 500.

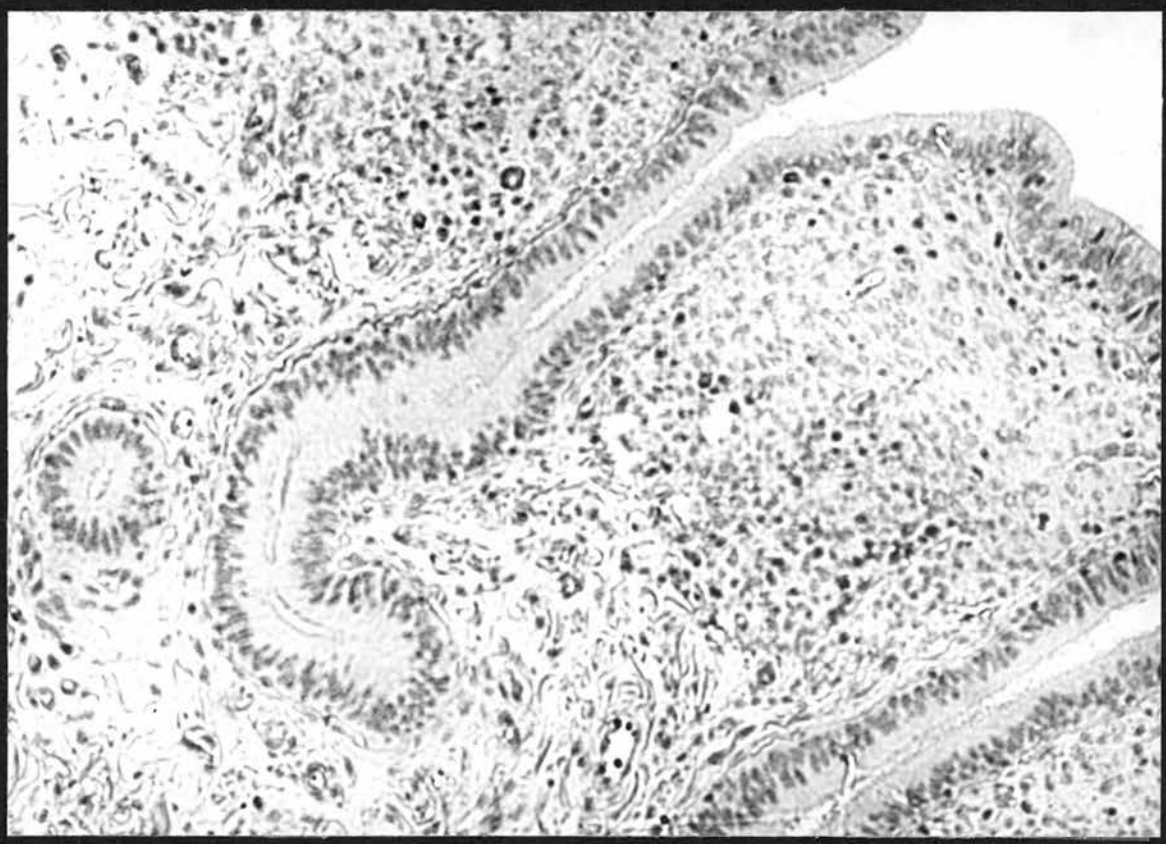


FIG. 9

Uterus - dioestrum.

The uterine epithelium is pseudostratified and active. The endometrial stroma is vascular and infiltrated with lymphocytes. Note intraepithelial lymphocytes.

PAS & H. X 500.

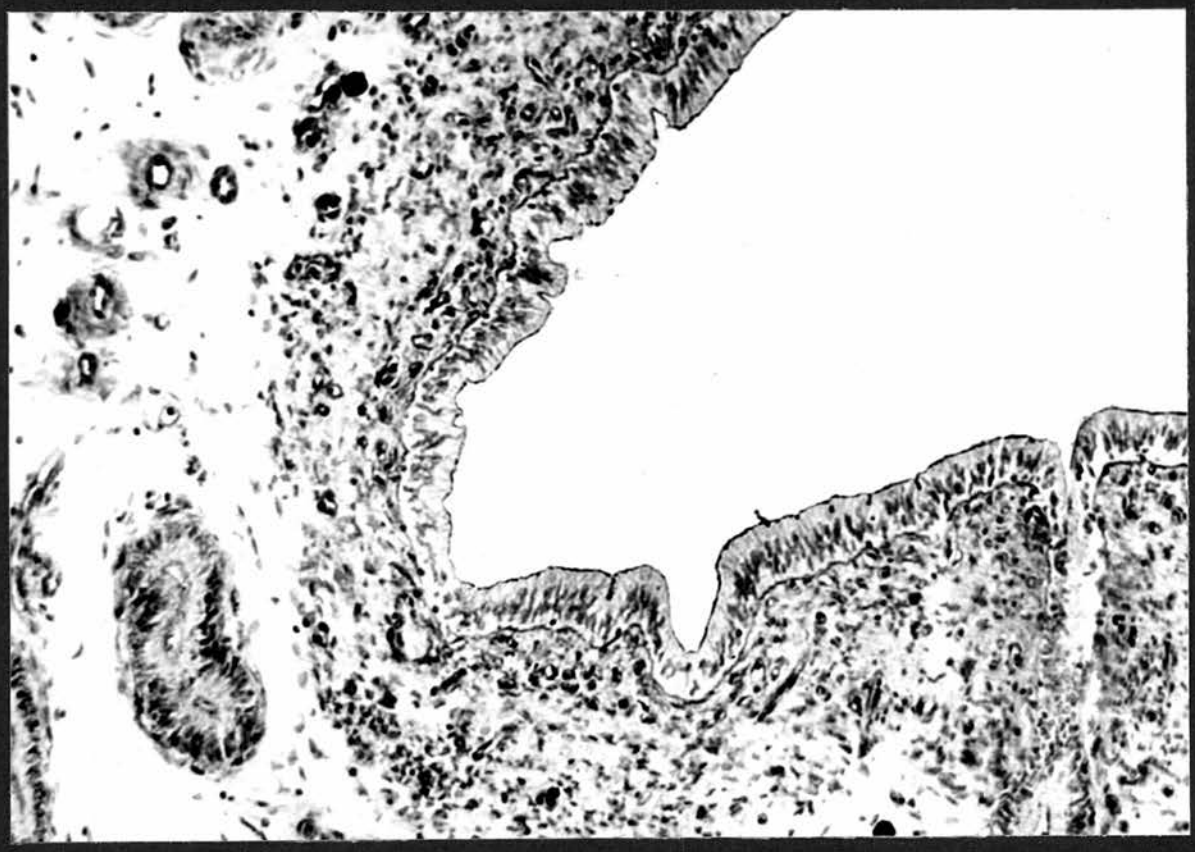


FIG. 10

Oviduct ampulla - late pro-oestrum.

The tubal epithelium shows PAS positive (diastase resistant) in the apical parts of the non-ciliated cells.

PAS & H. X 1000.

FIG. 11

Oviduct ampulla - late pro-oestrum.

The tubal epithelium shows Alcian blue substance in the apical parts of the non-ciliated cells.

Alcian blue and neutral red. X 1000.

FIG. 12

Oviduct ampulla - pro-oestrum. Freeze dried section. Alkaline phosphatase activity in the cilia of ciliated tubal cells. Phase contrast microphotograph.

Gomori's calcium-cobalt method. X 2000.

FIG. 13

Oviduct ampulla - metoestrum. Freeze dried section. Alkaline phosphatase activity at the distal border of some cells and in the lumen of the tube.

Gomori's calcium-cobalt method. X 500.

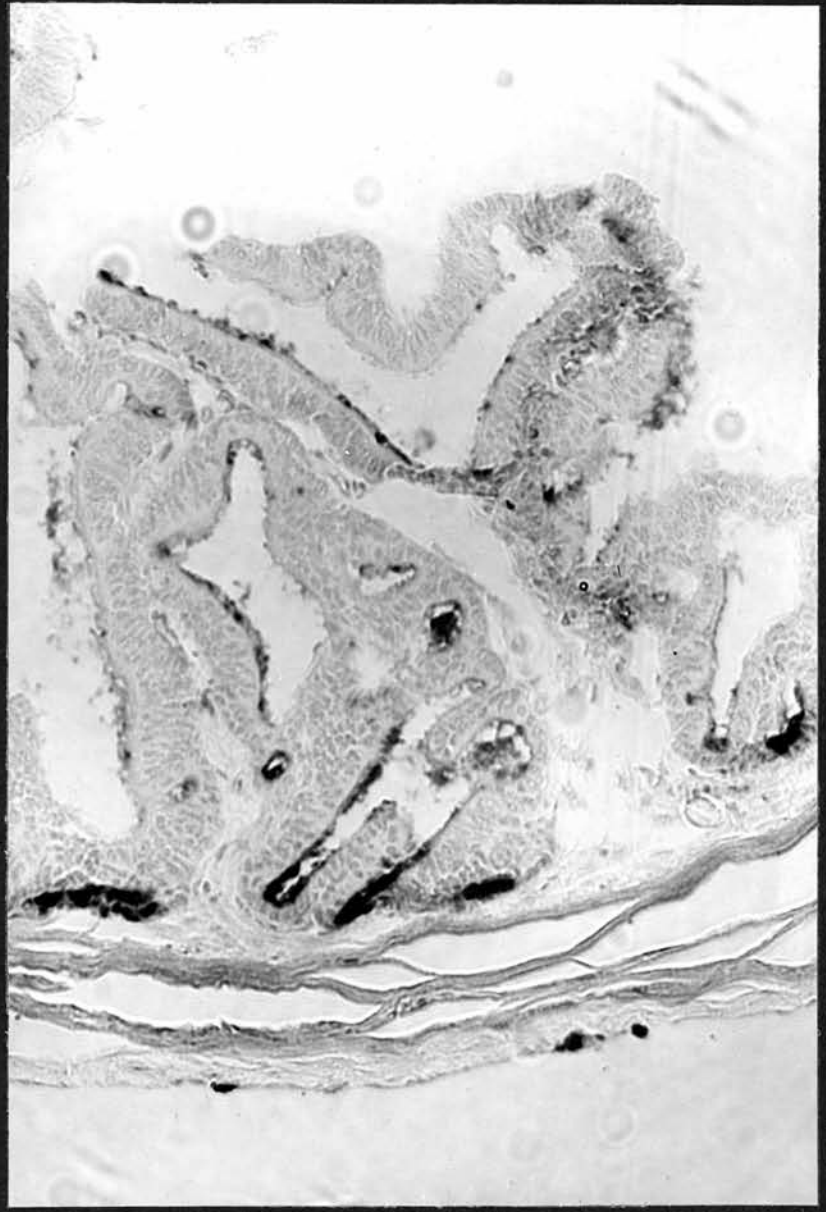


FIG. 14

Oviduct isthmus - dioestrum. Alcohol acetic acid formalin fixation.

Alkaline phosphatase activity at the distal border of the tubal epithelial cells. Present in all stages.

Gomori's calcium-cobalt method. X 500.

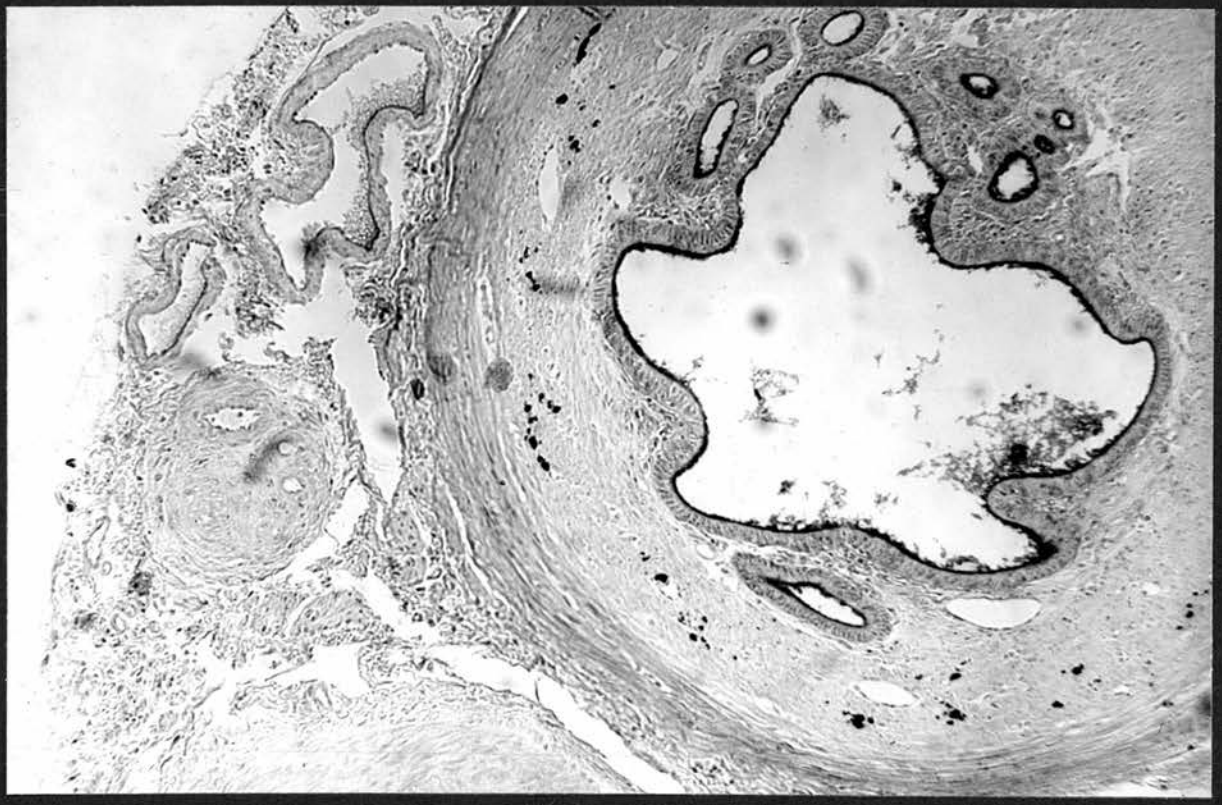


FIG. 15

Oviduct ampulla - metoestrum. Freeze dried section. Acid phosphatase activity in the apical parts of the tubal epithelial cells.

Gomori's lead nitrate method (modified). X 500.

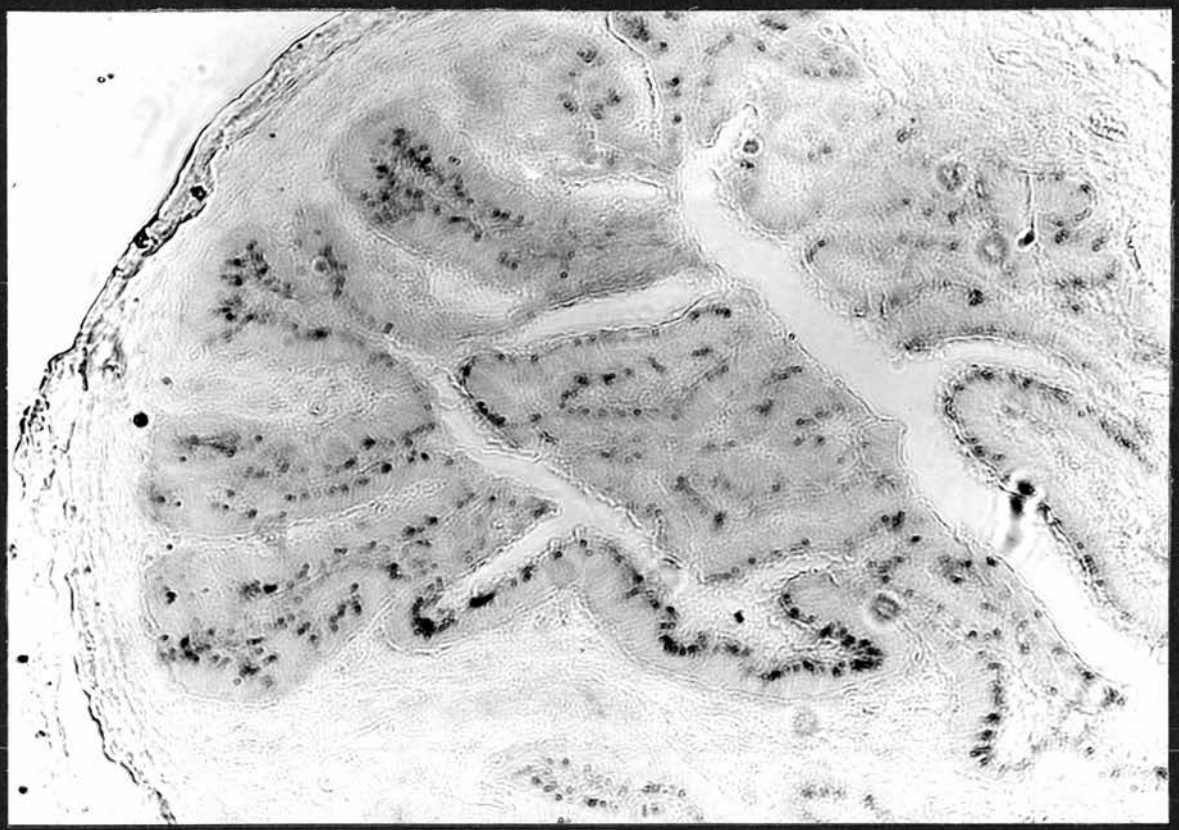


FIG. 16

Oviduct ampulla - metoestrum. Higher magnification of Fig. 15.

Acid phosphatase activity in the apical parts of the non-ciliated cells. Phase contrast microphotograph.

Gomori's lead nitrate method (modified). X 2000.

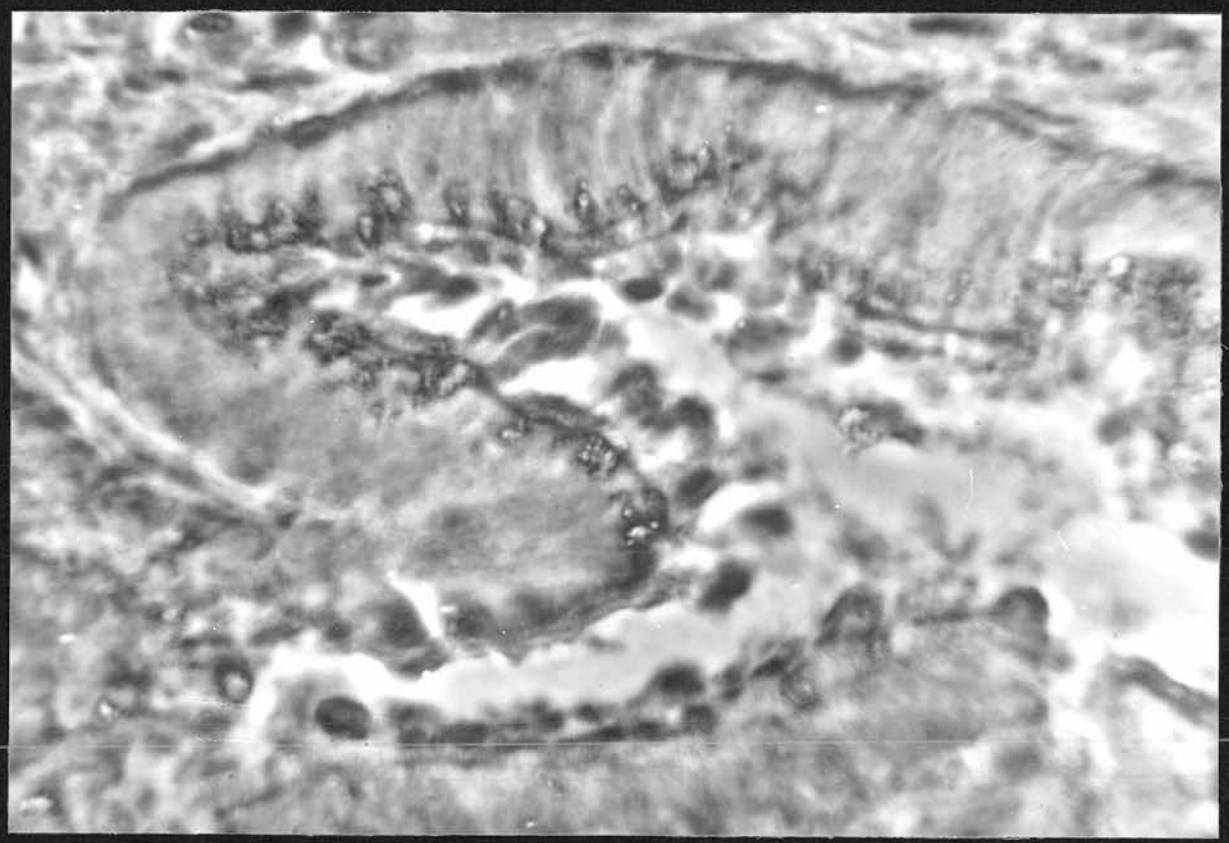


FIG. 17

Oviduct ampulla - pro-oestrum. Controlled chromation section.

Lipid droplets in the tubal epithelial cells and the subepithelial layers.

Sudan Black B method. X 500.

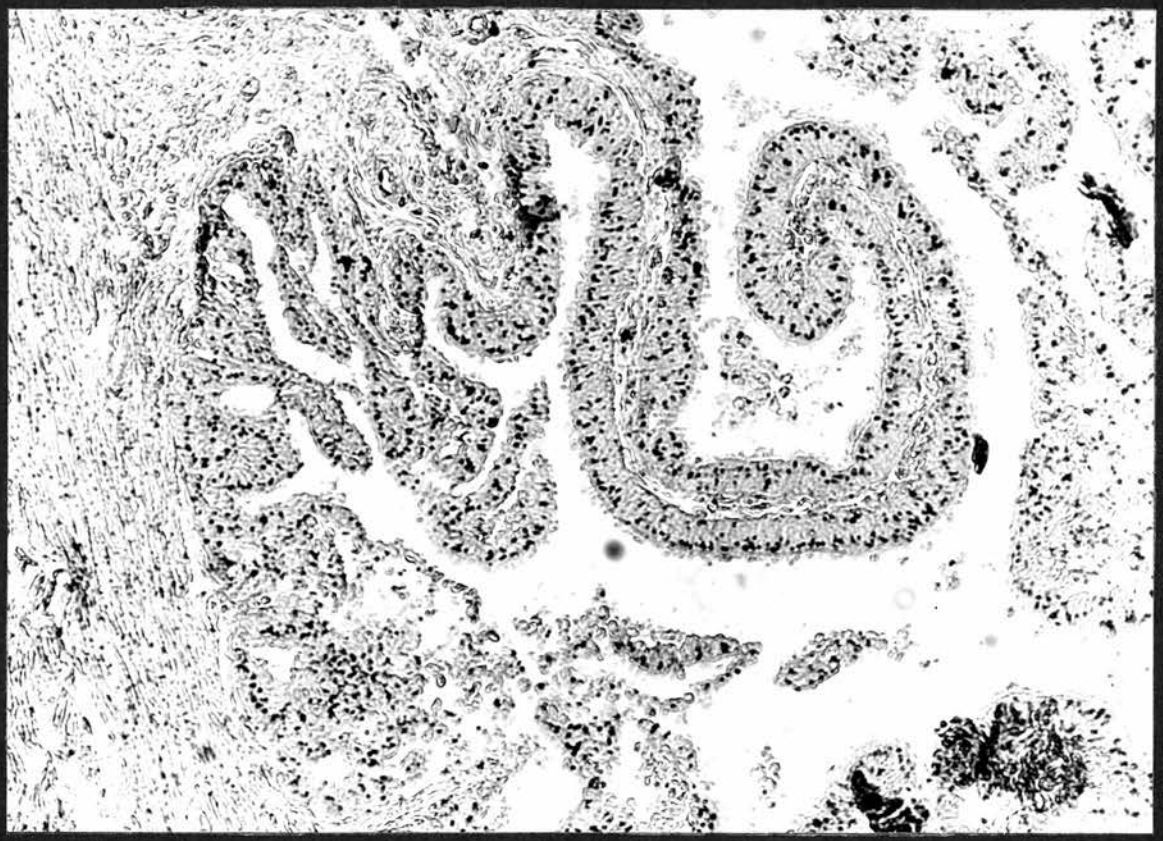


FIG. 18

Oviduct isthmus. Controlled chromation section.
Lipid droplets in the Golgi zone of the tubal cells.
Present at all stages.

Sudan Black B method. X 2000.

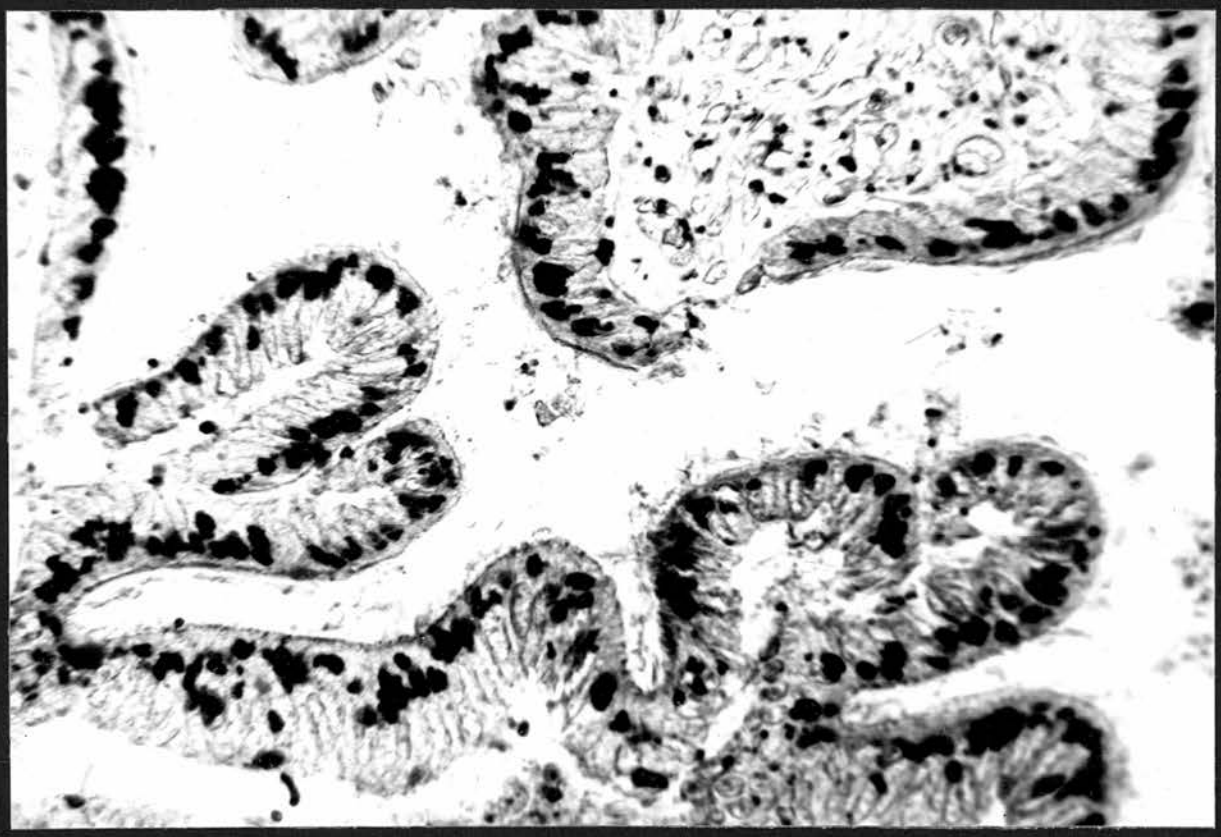


FIG. 19

Oviduct ampulla - metoestrum.

Ribonucleic acid in apical parts of the tubal cells.

Methyl Green Pyronin Y method. X 1000.

FIG. 20

Oviduct ampulla - early dioestrum. Neutral formalin fixation.

Traces of inorganic iron (Prussian blue reaction) along the free border of the tubal epithelial cells.

Scattered granules are present in epithelial cells and subepithelial layer.

Perl's method. X 1000.

FIG. 21

Uterus - dioestrum.

PAS positive reaction (diastase resistant) along the free border of the uterine epithelium. Also the basement membrane, the round cells in the endometrial stroma and the inner lining of the blood vessels show a similar reaction.

PAS & H. X 1000.

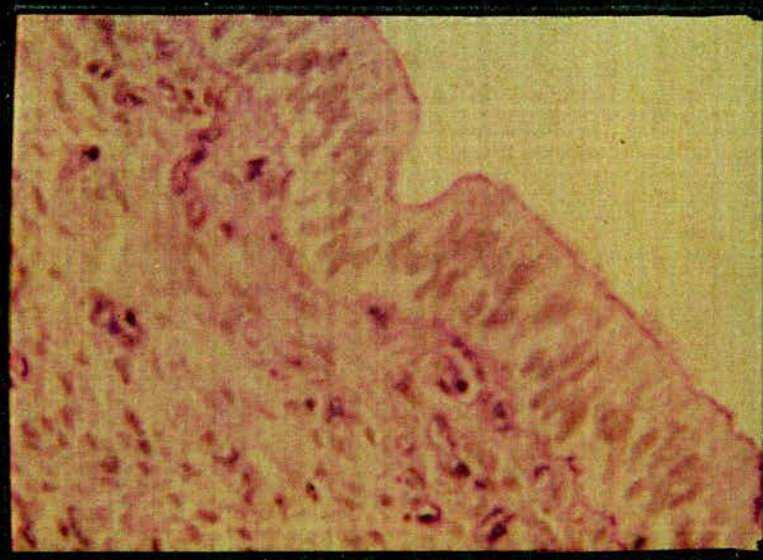


FIG. 22

Uterus - metoestrum.

Alkaline phosphatase activity at the free border of the uterine and glandular epithelium and within the lumen.

Gomori's calcium-cobalt method. X 500.

FIG. 23

Uterus - metoestrum. Neutral formalin fixation. Acid phosphatase activity almost limited to the lumen of the uterine glands.

Gomori's lead nitrate method (modified). X 500.

FIG. 24

Uterus - pro-oestrum. Controlled chromation section. Large lipid droplets in the apical parts of the uterine and glandular epithelial cells. Note the scattered droplets in the stroma.

Sudan Black B method. X 500.

FIG. 25

Uterus - dioestrum.

Ribonucleic acid in lymphocyte-like cells of endometrial stroma. Note part of a uterine gland is faintly stained.

Methyl Green Pyronin Y method. X 1000.

FIG. 26

Uterus - pro-oestrum. Neutral formalin fixation. Inorganic iron granules within the glandular epithelial cells. Note the diffuse Prussian blue reaction in the endometrial stroma.

Perl's method. X 1000.

FIG. 27

Oviduct ampulla - early pregnancy.

Irregular surface of the tubal epithelium. Note the pear-shaped protruding nuclei and the cytoplasmic projections.

H. & E. X 2000.

FIG. 28

Oviduct - mid pregnancy.

The irregular surface of the tubal epithelium and the cytoplasmic projections with or without nuclei are characteristic features of the oviduct during most of gestation.

H. & E. X 1000.

FIG. 29

Uterus - before implantation.

The uterine epithelium is tall columnar and a few lymphocytes are intracellular. The subepithelial layer is hypertrophied, vascular and infiltrated by lymphocytes.

H. & E. X 500.

N.B. The chorionic epithelium consists of a single layer of chorionic cells and the binucleate cells have not appeared yet.

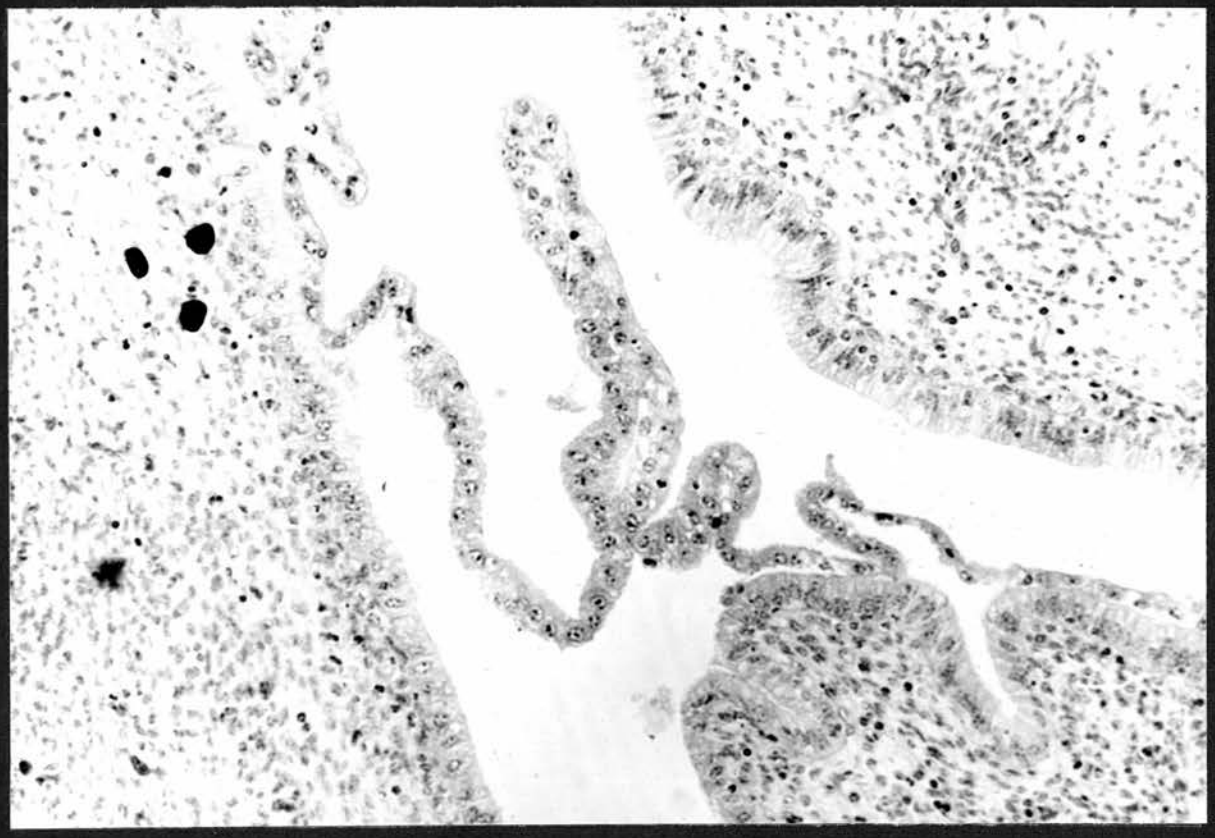


FIG. 30

Gravid uterus - embryo 5 mm. C.R.L.

The endometrium is denuded of its surface epithelium. The stroma is infiltrated with lymphocytes migrating towards the lumen of the glands. Note aggregates in the lumen of some glands.

H. & E. X 500.

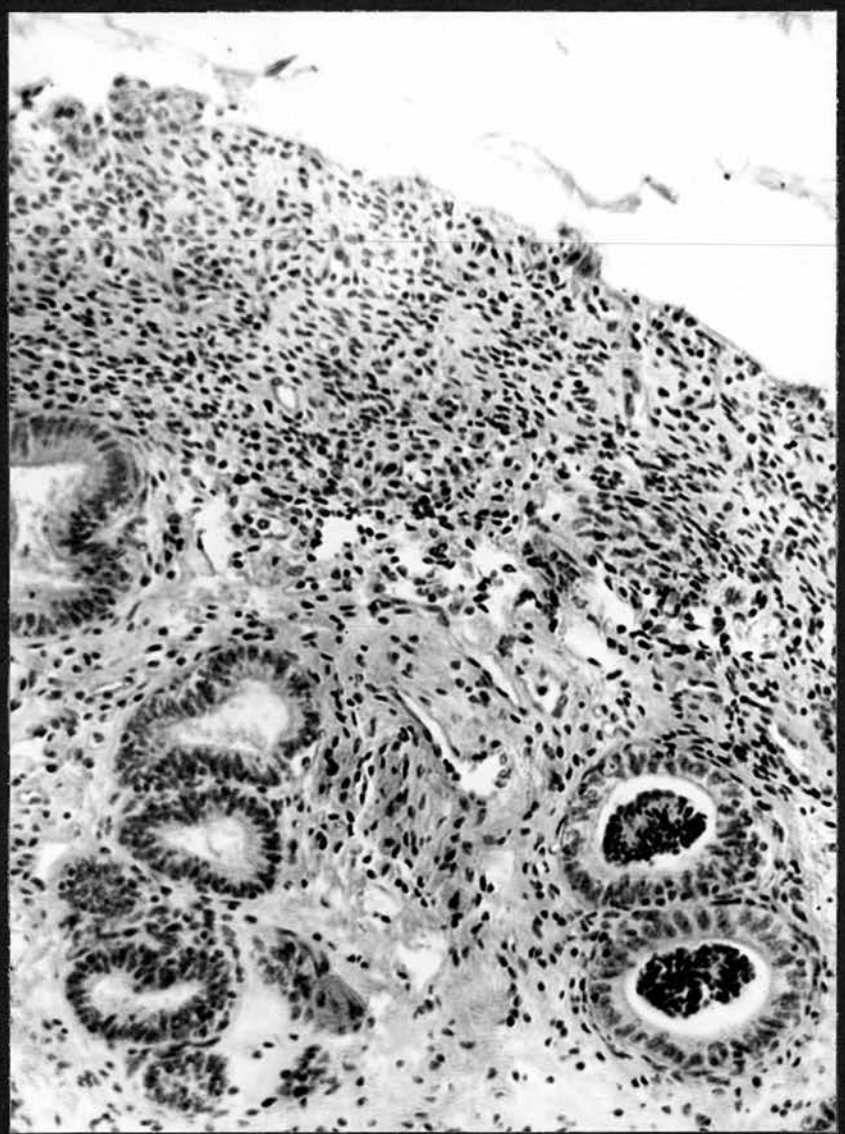


FIG. 31

Gravid horn - embryo 16 mm. C.R.L.

Uterine epithelium is intact. Note the very tall columnar cells and the intraepithelial infiltration of lymphocytes.

H. & E. X 500.

FIG. 32

Gravid uterus - intercaruncular area - embryo 20 mm. C.R.L.

Uterine epithelium is intact at junctional zone between caruncular and intercaruncular areas. Note the intra-epithelial lymphocyte-like cells containing eosinophilic granules.

H. & E. X 2000.

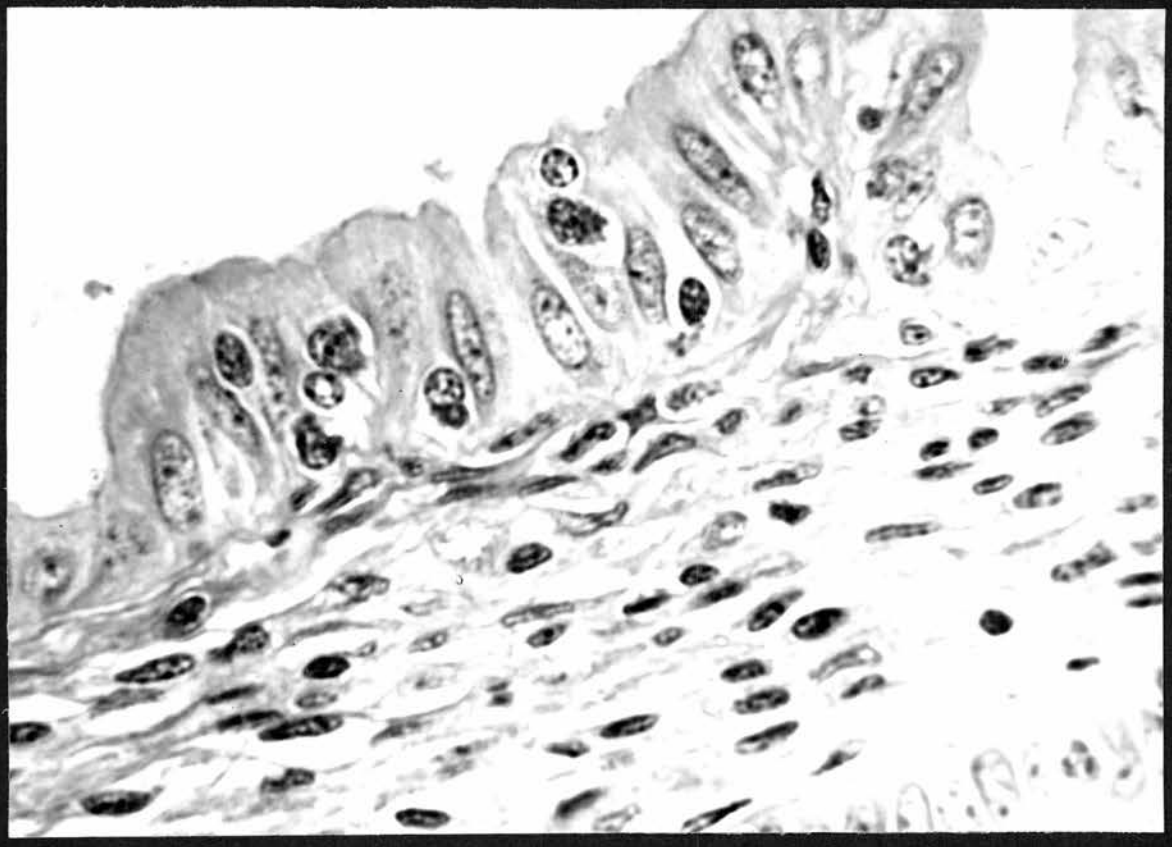


FIG. 33

Gravid uterus - caruncular area - embryo 20 mm. C.R.L. Tracks of degenerative tissue at right angles to the surface.

H. & E. X 500.

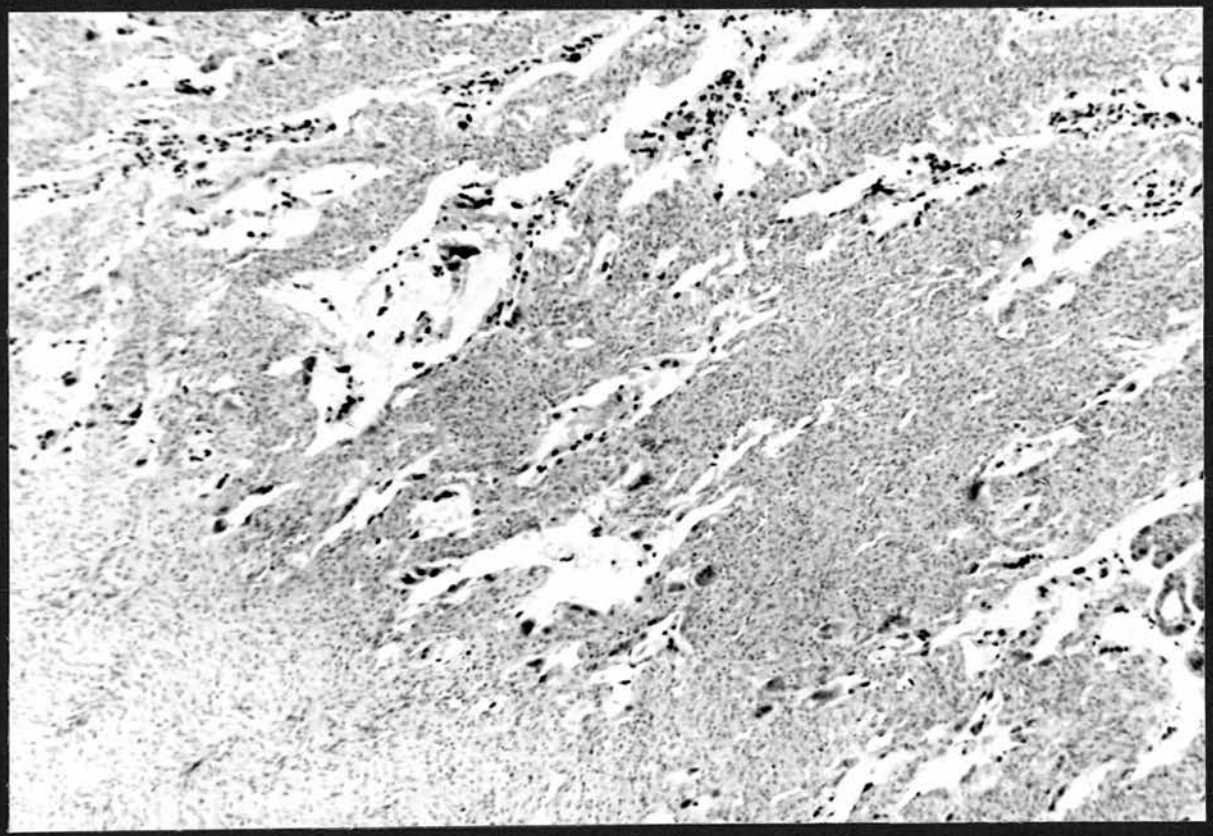


FIG. 34

Placenta - cotyledonary area - embryo 25 mm. C.R.L. Note binucleate and multinucleate giant cells on the surface of the primitive crypts. Note also folds of chorionic epithelium occupying the evacuated crypts.

H. & E. X 500.

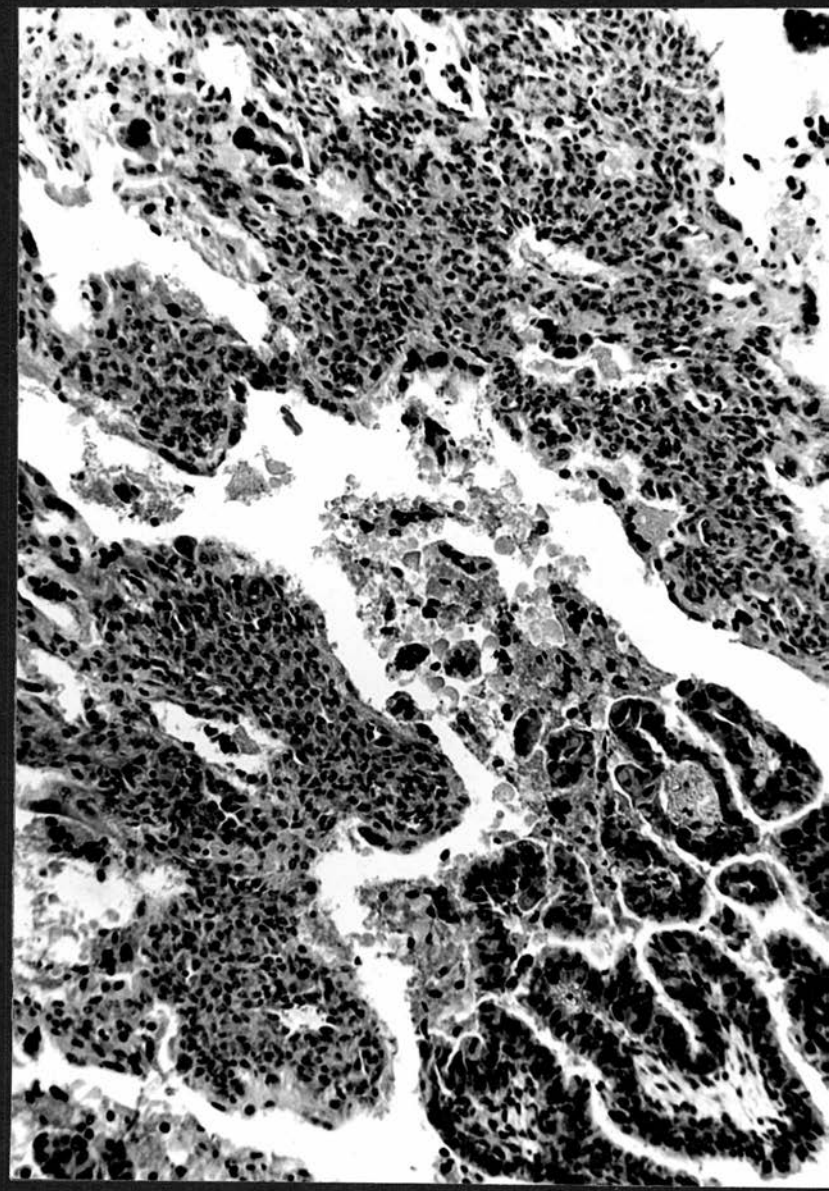


FIG. 35

Placenta - intercaruncular area - embryo 25 mm. C.R.L. Part of uterine epithelium is intact and a neighbouring part is replaced by the dark giant cells of the trophoblast. The chorionic epithelium is on the left side of the figure.

H. & E. X 1600.

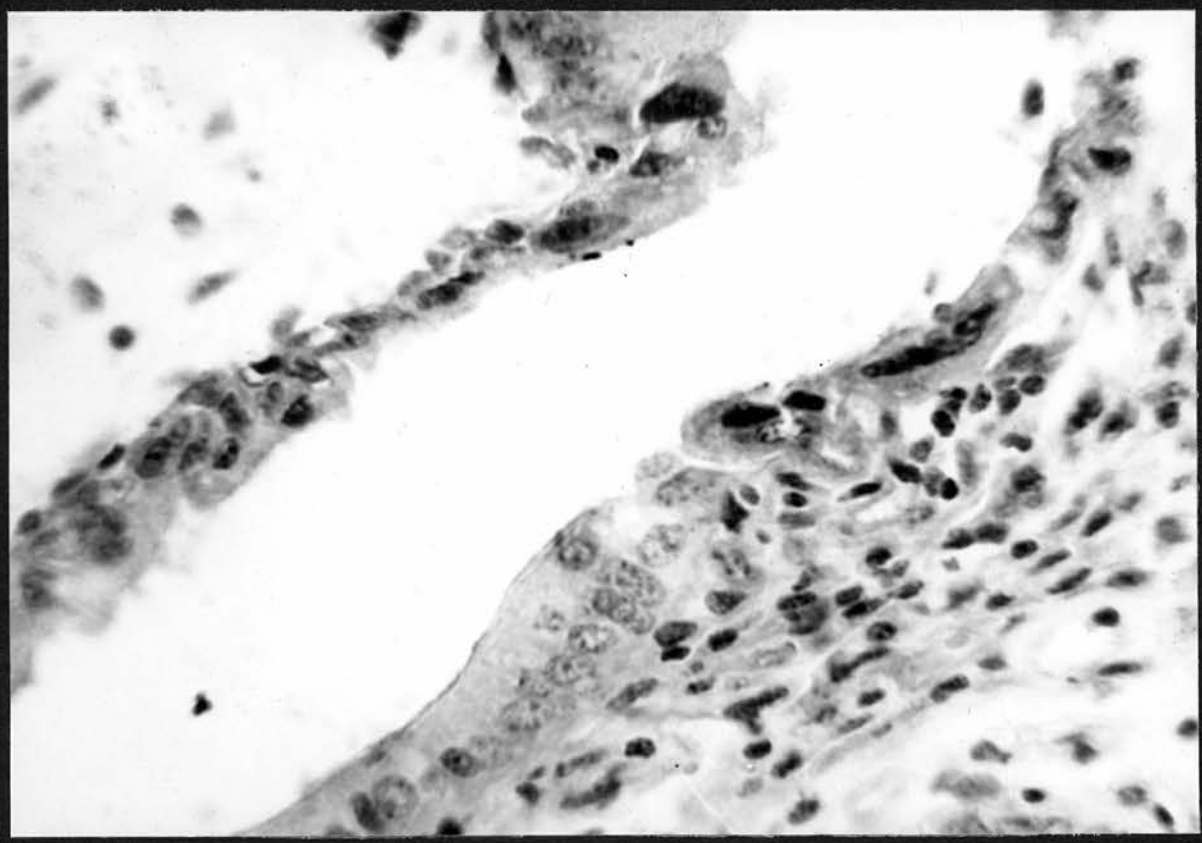


FIG. 36

Placenta - cotyledonary area - foetus 60 mm. C.R.L.
The maternal crypts are lined by flattened multinucleate giant cells discontinued in some places. The foetal villi are lined by chorionic epithelium which includes binucleate cells.

H. & E. X 500.

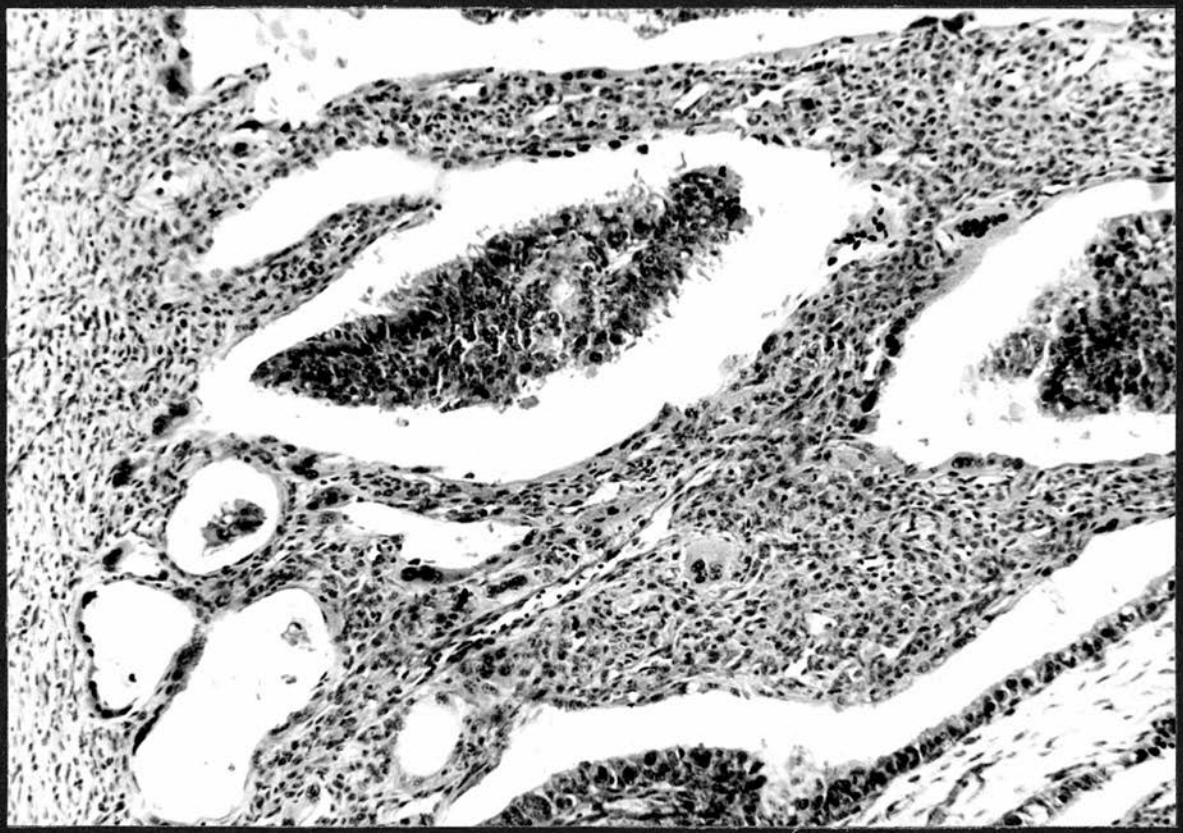


FIG. 37

Placenta - intercotyledonary area - foetus 60 mm. C.R.L. Note chorionic "areola" opposite the opening of a uterine gland. Note also debris between the two invaginations.

H. & E. X 800.

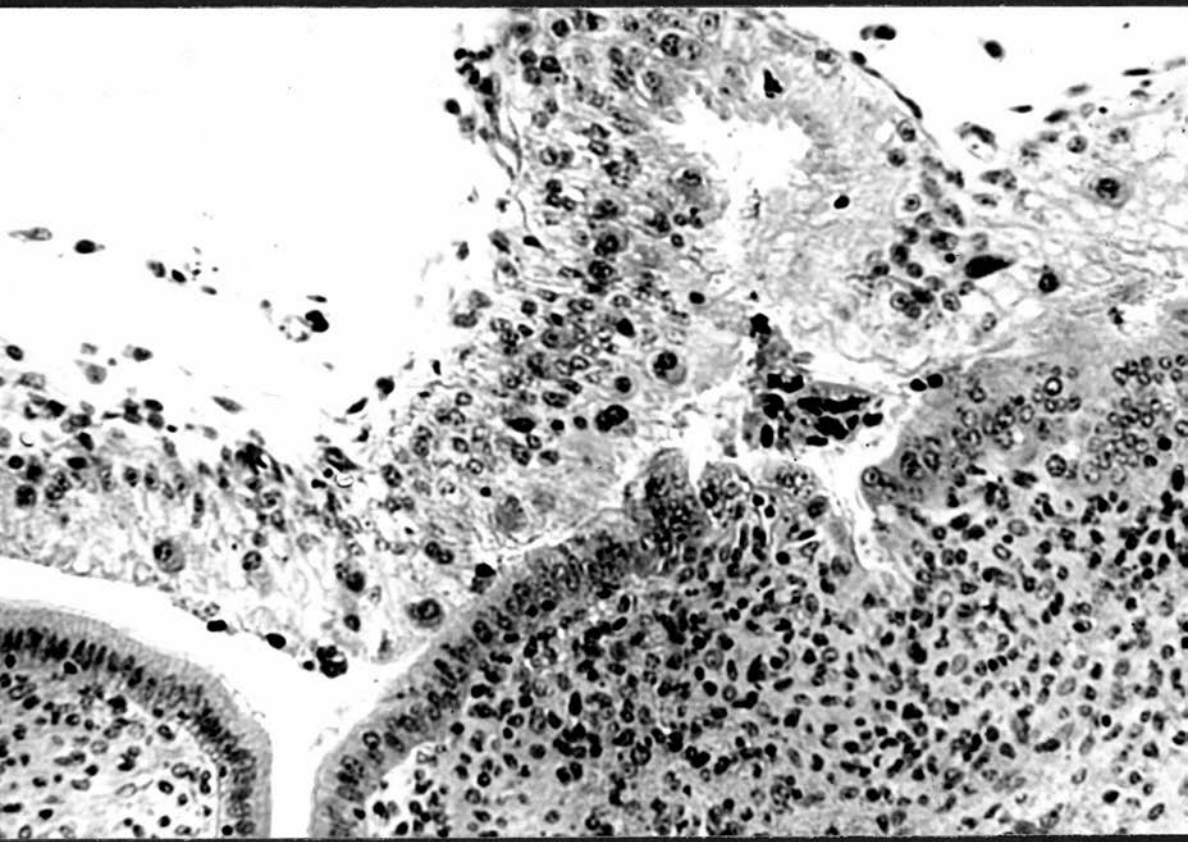


FIG. 38

Placenta - fully developed placentome (80-90 days approx.)

Extravasation of blood between a tip of a maternal septum and a base of a villus. Note red blood corpuscles and pigments within the tall columnar chorionic cells (phagocytosis).

Masson's trichrome stain. X 1000.

FIG. 39

Placenta - fully developed placentome (80-90 days approx.)

Slender maternal septa with bud-like branches containing blood vessels. Chorionic villi enveloping the vascular maternal septa. Note the tall columnar epithelium at the bases of the villi.

Masson's trichrome stain. X 200.

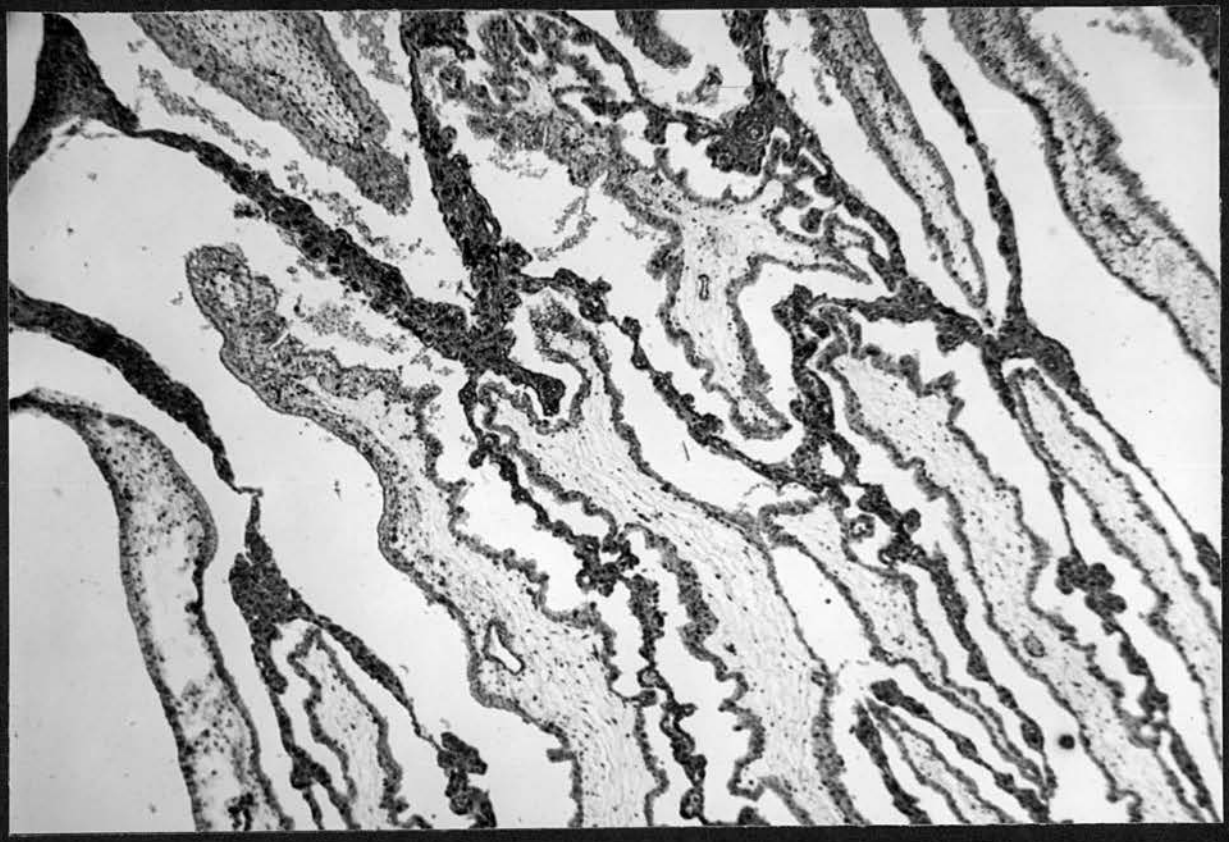


FIG. 40

Placenta - gravid horn (120 days pregnancy approx.). Hypertrophied and active uterine glands and chorionic folds with allantoic vessels. Note the wide diameter of the uterine glands.

H. & E. X 200.

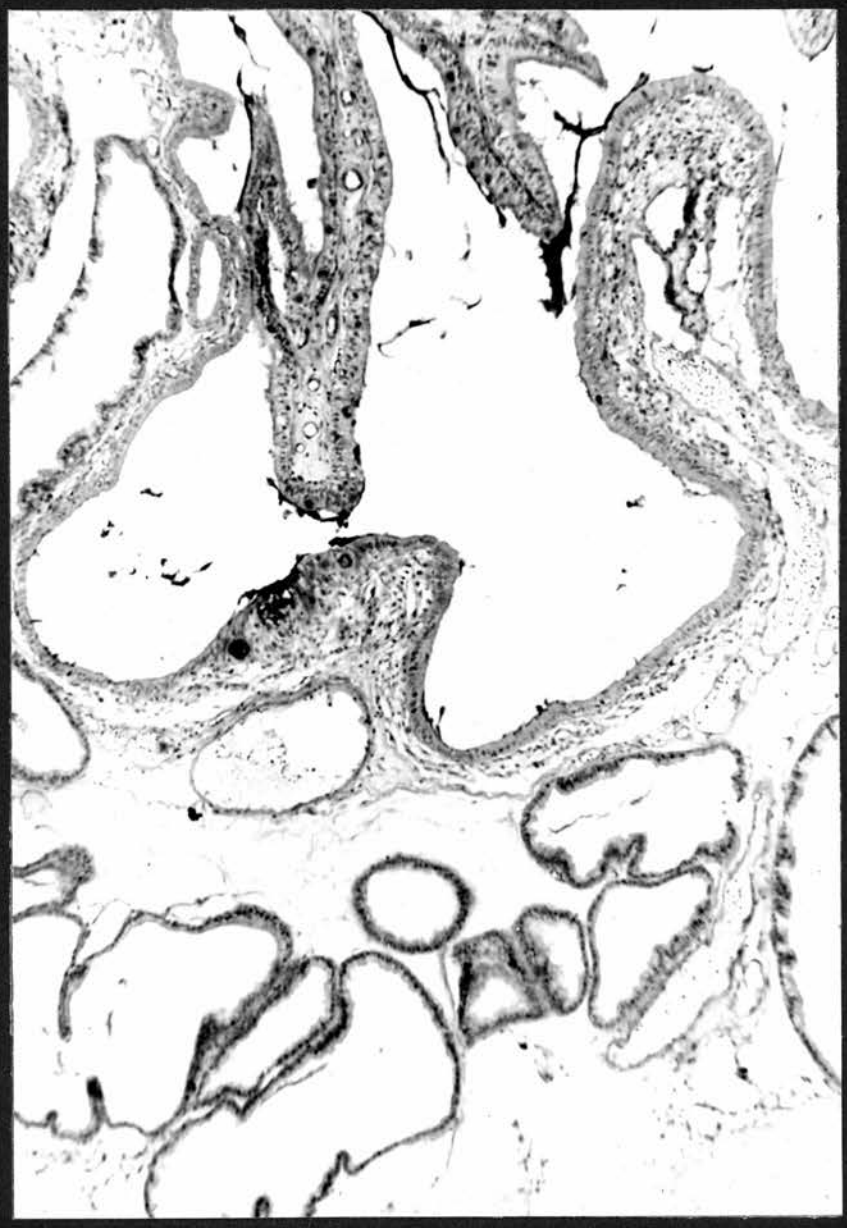


FIG. 41

Placenta - tapering part of a gravid horn (120 days pregnancy approx.).

Tall columnar epithelium of the uterus (bottom) and secretion and debris in the lumen (PAS positive).

Note the necrotic epithelium of the chorion and its pycnotic nuclei (upper part of the figure).

PAS & H. X 500.

FIG. 42

Gravid uterus - caruncular area - embryo 16 mm. C.R.L.
A caruncular area denuded of its surface epithelium.
Note melanocytes in the endometrial stroma and free melanin granules near the denuded surface.

H. & E. X 2000.

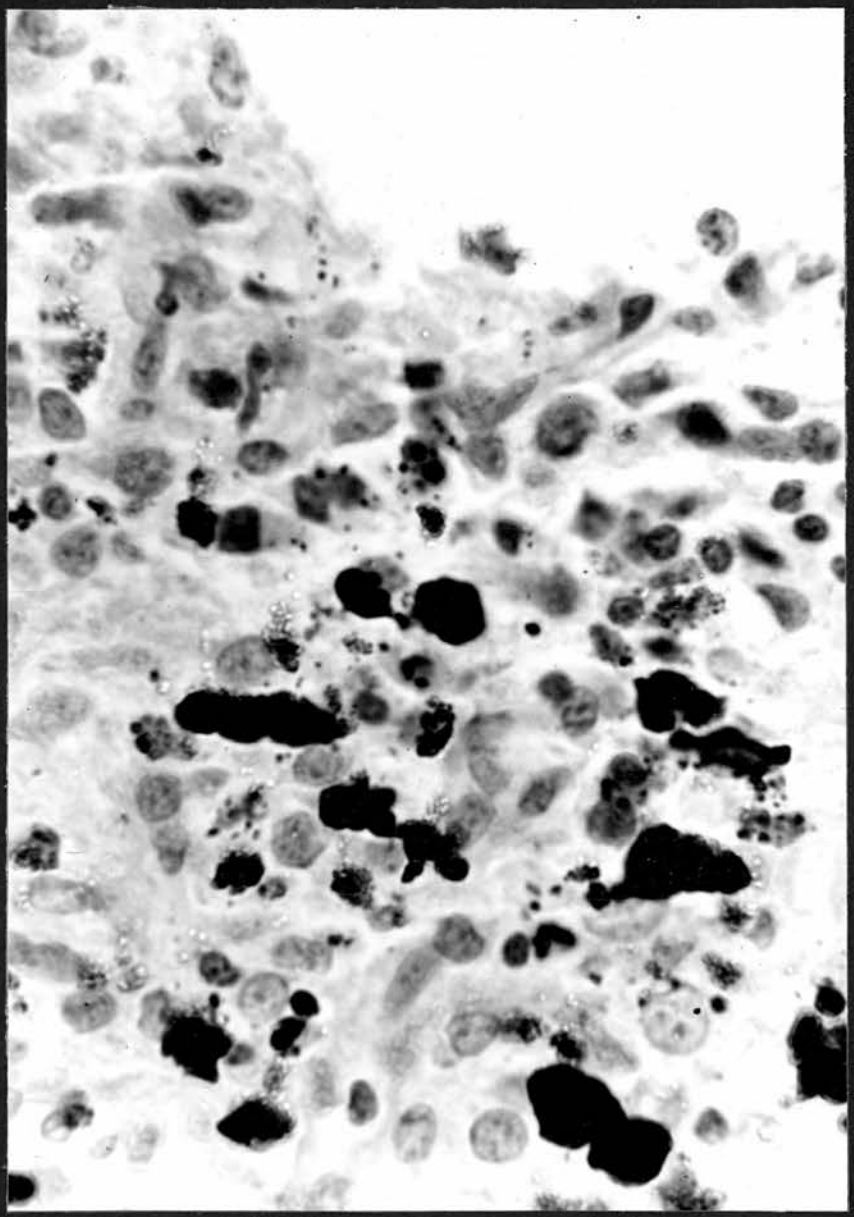


FIG. 43

Placenta - placentome of late pregnancy (140 days pregnancy approx.).

Extravasated blood near a base of a villus. Note the red blood corpuscles and pigment granules in the chorionic epithelial cells.

H. & E. X 2000.

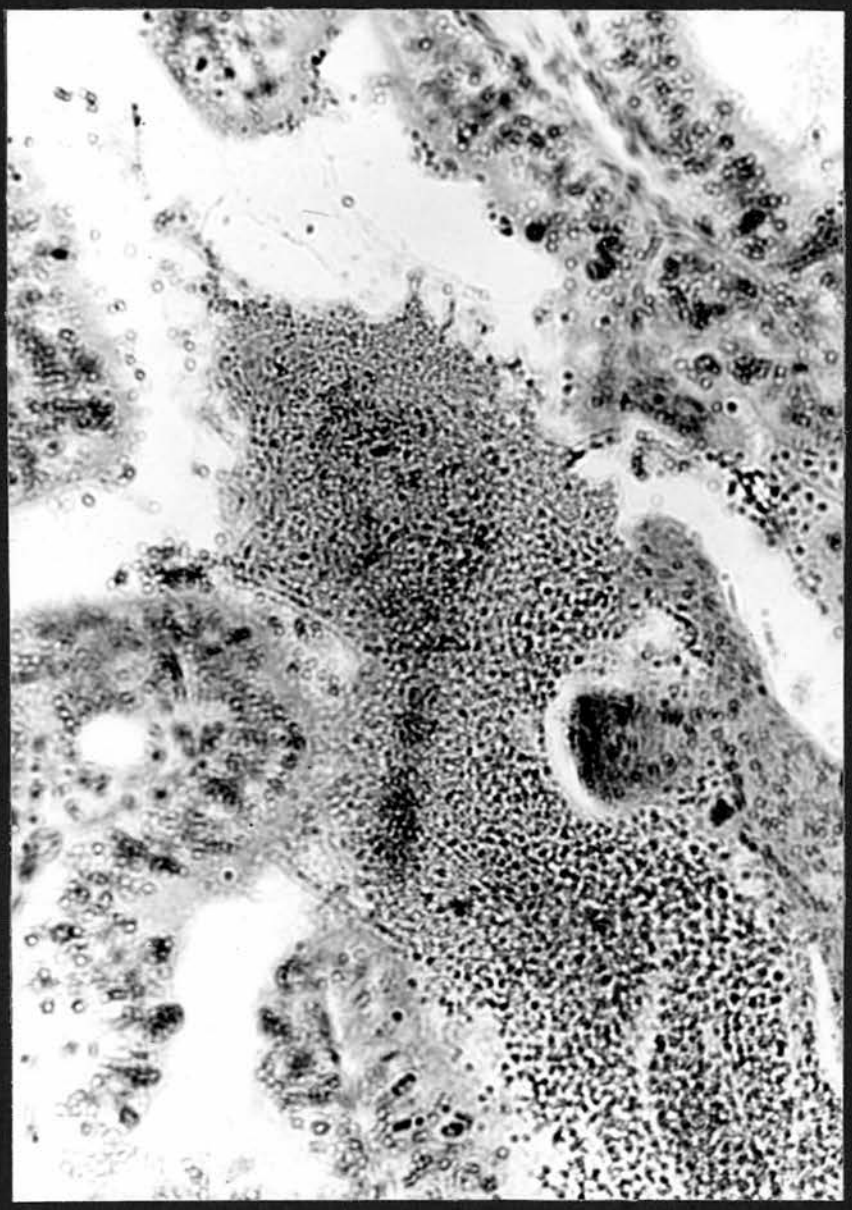


FIG. 44

Placenta - fully developed placentome (80-90 days pregnancy approx.).

Hypertrophied endothelial lining of maternal blood vessels covered by a coat of argyrophil fibrils.

Wilder's silver oxide method. X 1600.

FIG. 45

Oviduct ampulla - embryo 60 mm. C.R.L.

Glycogen granules within the tubal epithelium and especially in the cytoplasmic projections.

Best's carmine method (diastase controlled). X 800.

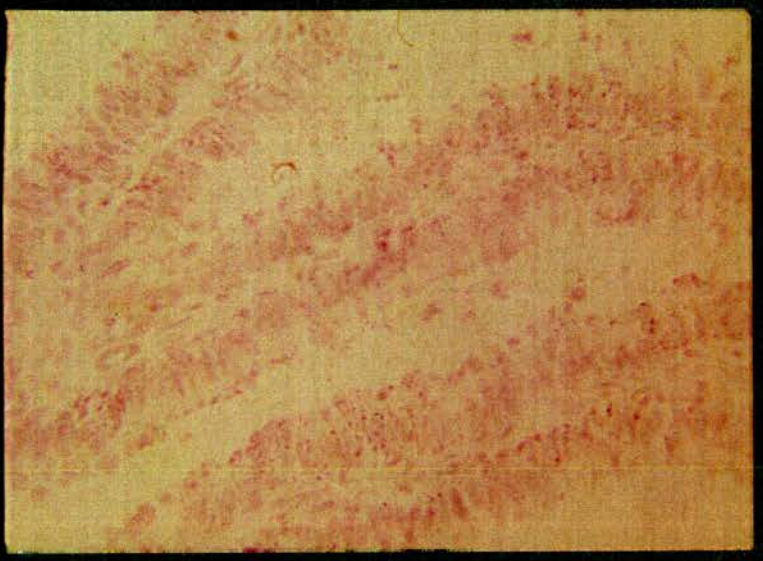


FIG. 46

Oviduct ampulla (80-90 days pregnancy approx.). Freeze dried section.

Alkaline phosphatase activity along the free border of the tubal epithelium and the inner lining of the subepithelial blood vessels.

Gomori's calcium-cobalt method. X 500.

FIG. 47

Oviduct isthmus. Freeze dried section. Alkaline phosphatase activity at the free border of the tubal epithelium (present throughout most of gestation). Note pigment cells in subepithelial and muscular layers.

Gomori's calcium-cobalt method. X 500.

FIG. 48

Oviduct ampulla - embryo 60 mm. C.R.L. Freeze dried section.

Acid phosphatase activity in the apical parts of the tubal secreting cells.

Gomori's lead nitrate method (modified). X 500.

FIG. 49

Oviduct isthmus. Freeze dried section.

Acid phosphatase activity in the distal parts of most tubal cells.

Gomori's lead nitrate method (modified). X 500.

FIG. 50

Oviduct ampulla - embryo 45 mm. C.R.L. Controlled chromation section.

Lipid droplets in supranuclear parts of the cells and in the cytoplasmic projections.

Sudan Black B method. X 2000.

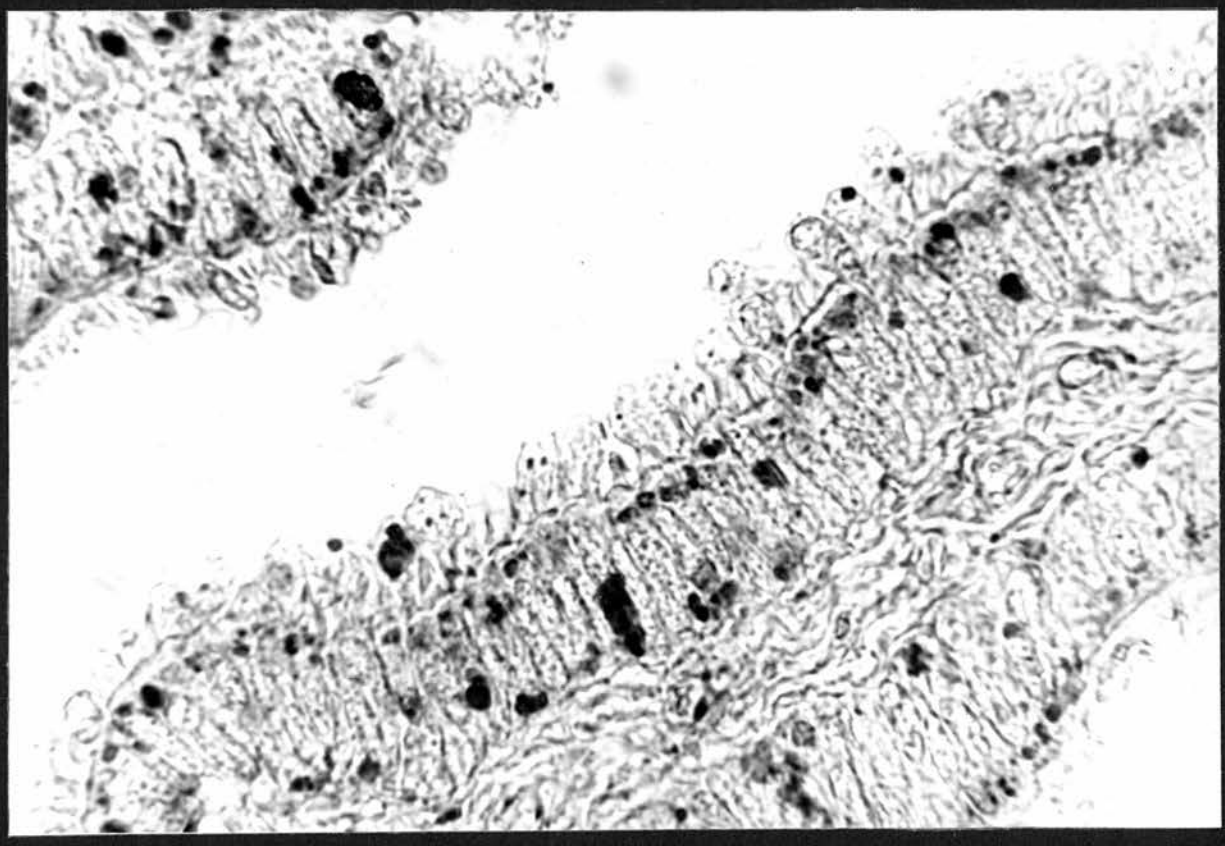


FIG. 51

Placenta - fully developed placentome (120 days pregnancy approx.). Freeze substitution section. Large glycogen granules in the wall of maternal blood vessel. The pink staining of the background is diastase resistant.

PAS & H. (diastase controlled). X 1000.

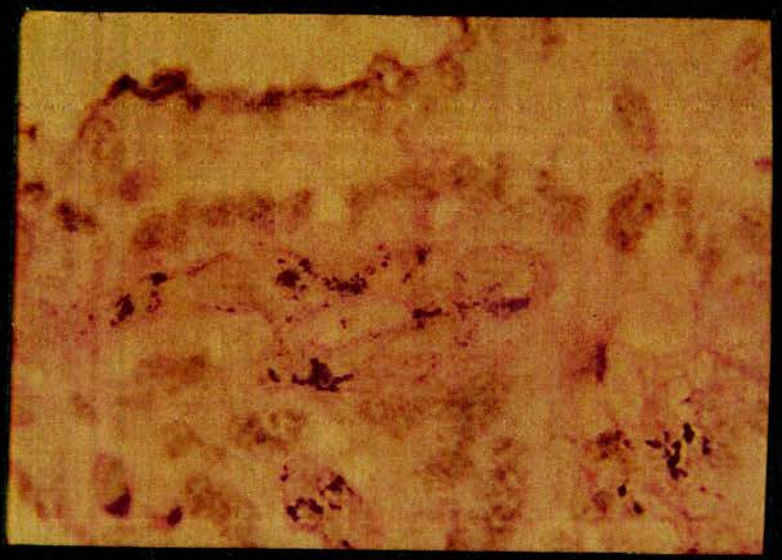


FIG. 52

Placenta - base of a villus (embryo 56 mm. C.R.L.). Glycogen granules in the wall of a foetal blood vessel and within the chorionic cells.

Best's carmine method. X 800.

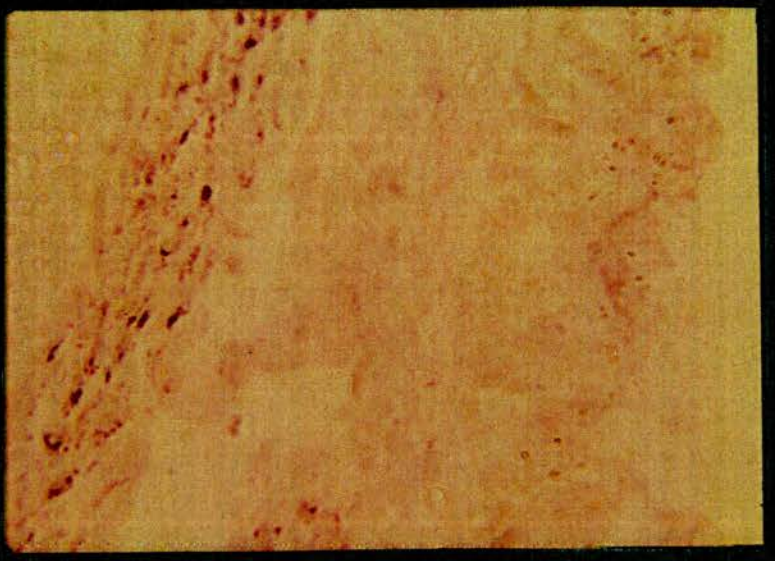


FIG. 53

Placenta - junctional zone of a placentome (embryo 60 mm. C.R.L.). AAF fixation.

Binucleate cells with eccentric nuclei and PAS positive cytoplasm (diastase resistant). Note the similar position of the binucleate cell on the maternal side.

PAS & H. (diastase controlled). X 1000.

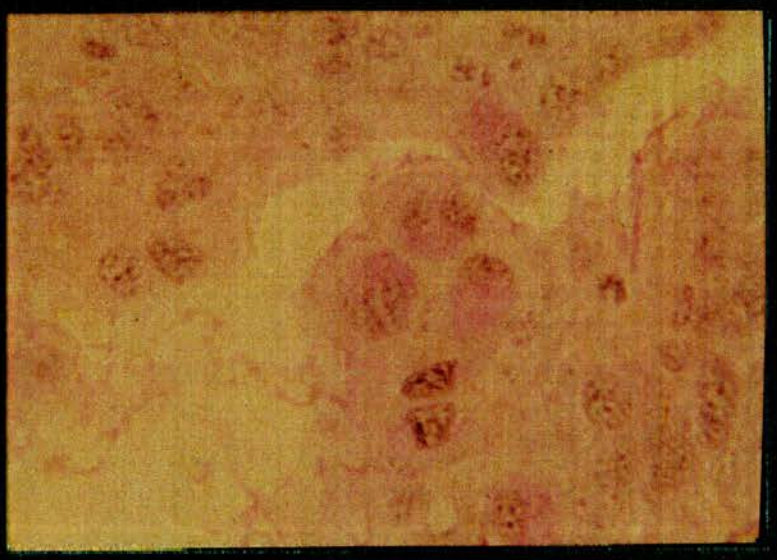


FIG. 54

Placenta - junctional zone of a placentome (embryo 60 mm. C.R.L.). Freeze dried section. Binucleate cells on foetal side showing even distribution of PAS positive substance (diastase resistant) excepting one cell where the nuclei are eccentric. Note also PAS positivity of the maternal vessel wall after diastase digestion.

PAS & H. (after diastase digestion). X 1000.

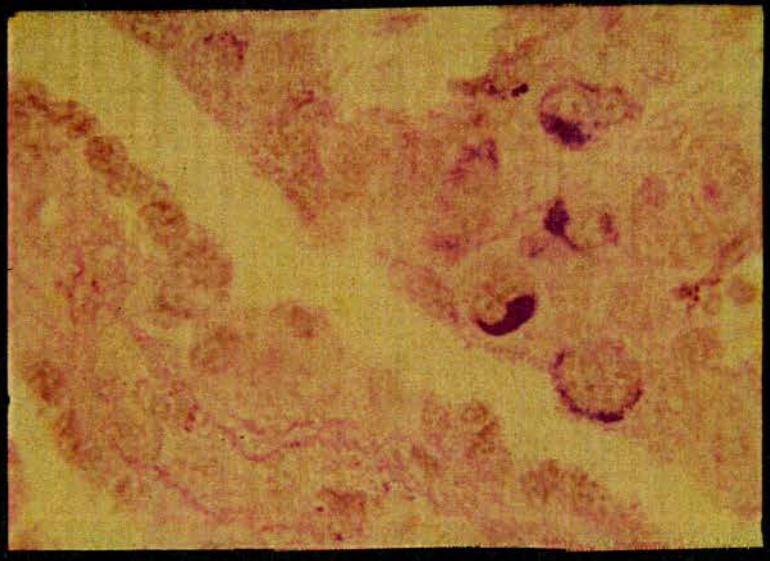


FIG. 55

Placenta - parts of cotyledonary and intercotyledonary areas (embryo 45 mm. C.R.L.). AAF fixation.
Alkaline phosphatase activity at:

the distal border of the uterine and glandular epithelium,

the syncytial lining of the maternal crypts,

the binucleate cells of the trophoblast,

the border of the chorionic cells, and

the inner lining of the blood vessels.

(Compare with next figure).

Gomori's calcium-cobalt method. X 360.

FIG. 56

Placenta - fully developed placentome (120 days pregnancy approx.). Freeze dried section. Alkaline phosphatase activity confined to the peripheral parts of the binucleate cells and the inner lining of the blood vessels.

Gomori's calcium-cobalt method. X 500.

FIG. 57

Gravid uterus - caruncular area (embryo 60 mm. C.R.L.).
AAF fixation.
Alkaline phosphatase activity in the inner lining of the blood vessels of the deep stroma and in the surface lining of the caruncle.

Gomori's calcium-cobalt method. X 200.

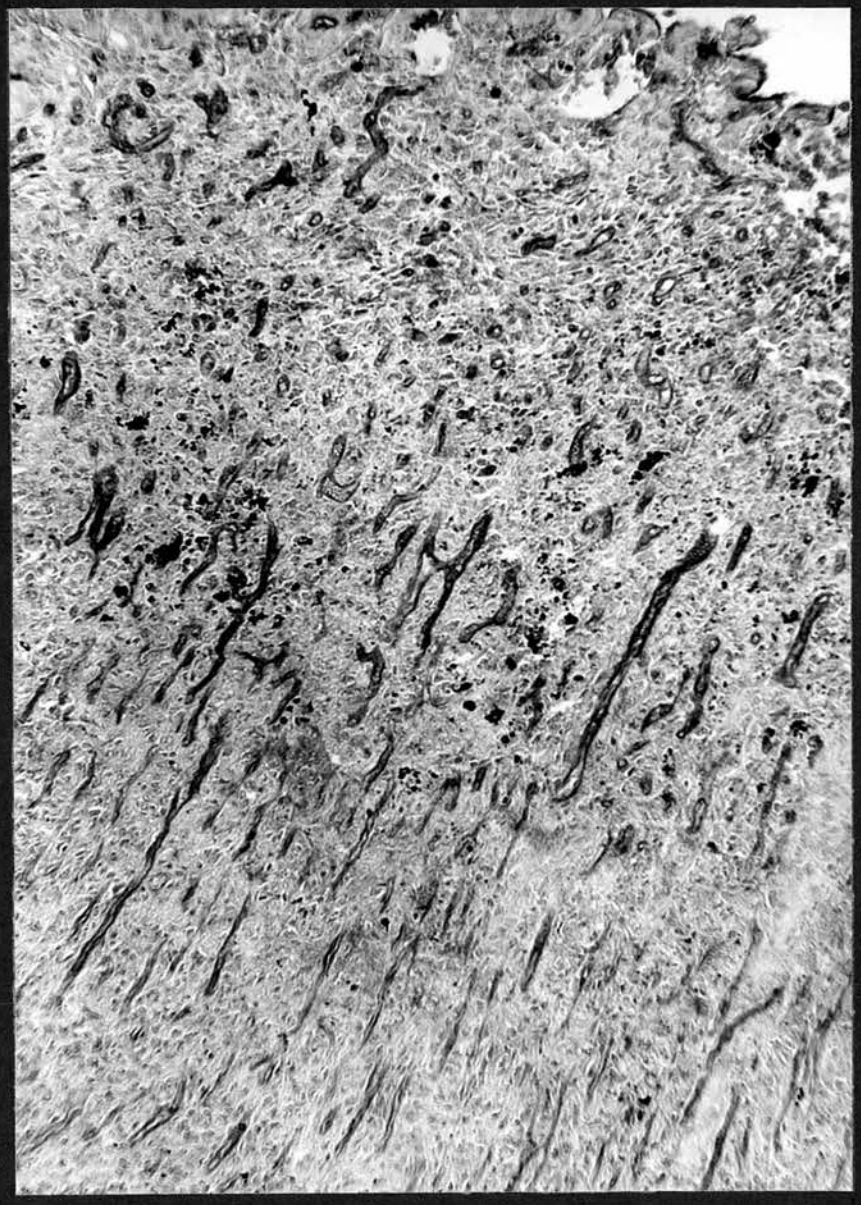


FIG. 58

Placenta - intercaruncular area (80-90 days pregnancy approx.). Freeze dried section.

Acid phosphatase activity in the glandular epithelial cells and their lumina.

Gomori's lead nitrate method (modified). X 500.

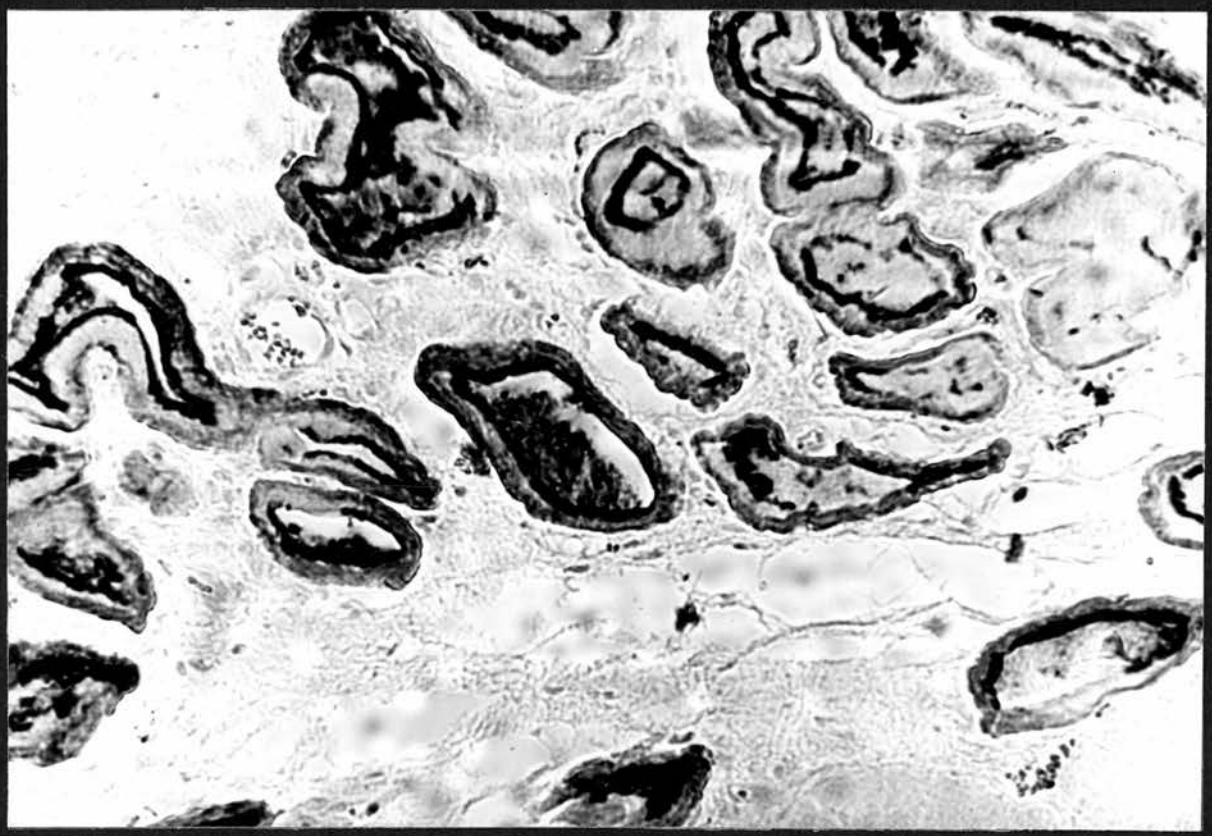


FIG. 59

Placenta - intercotyledonary area (120 days pregnancy approx.). Freeze dried section.

Acid phosphatase activity in:

the apical parts of the uterine epithelium,

the glandular cells,

the lumen between the foetal and maternal sides, and

the binucleate cells of the trophoblast.

Gomori's lead nitrate method (modified). X 360.

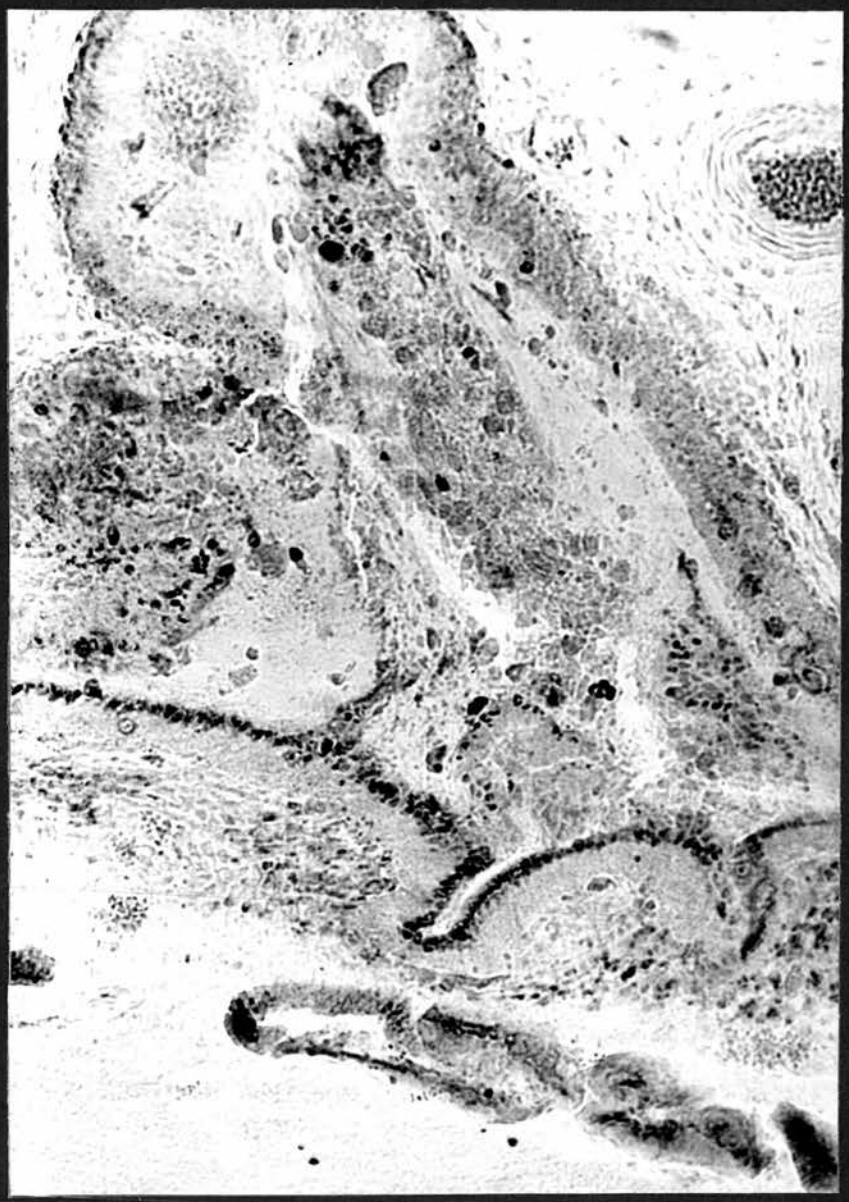


FIG. 60

Placenta - cotyledonary area (foetus 100 mm. C.R.L.).

Freeze dried section.

Acid phosphatase activity in:

the binucleate cells,

the borders of the other chorionic cells, and

the inner lining of the blood vessels.

Note that the activity is central (compare with Fig.56).

Note also that the activity is more in the maternal than in the foetal tissues.

Gomori's lead nitrate method (modified). X 500.

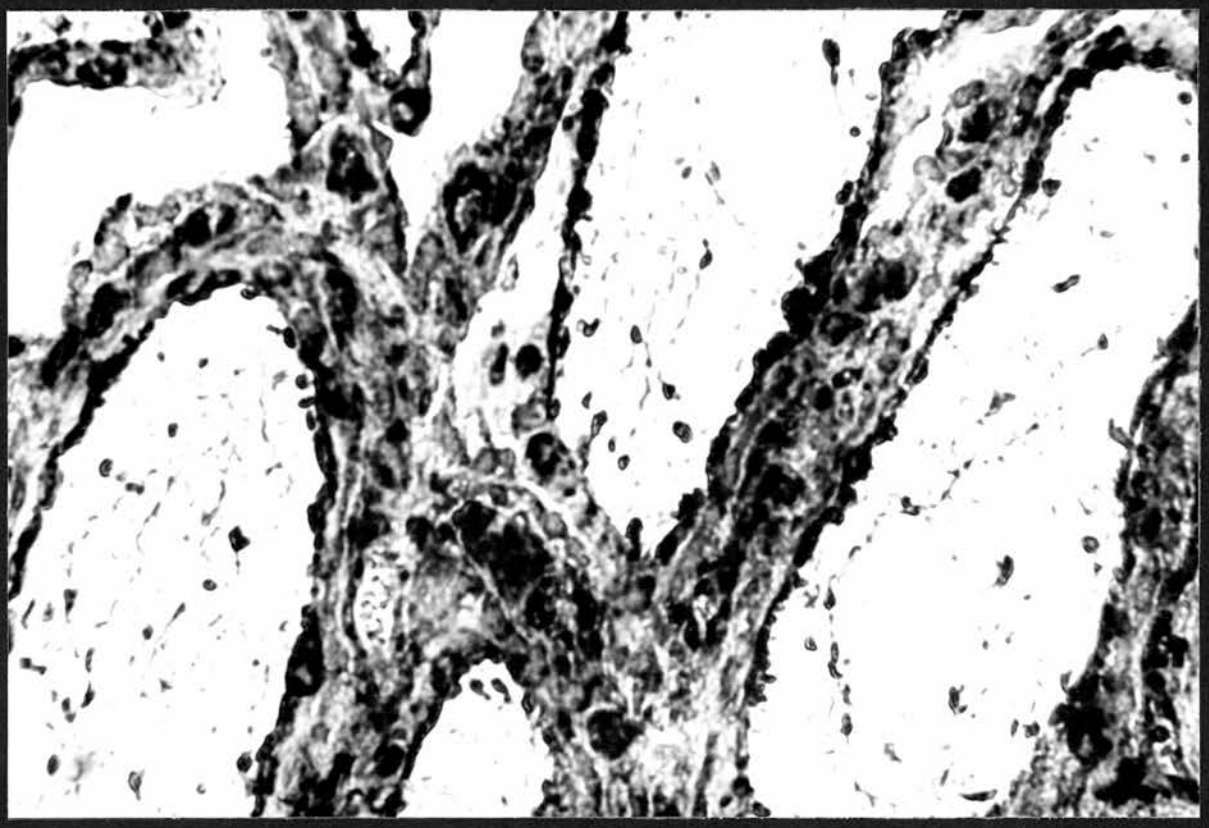


FIG. 61

Gravid uterus - intercaruncular area (embryo 60 mm. C.R.L.). Controlled chromation section. Lipid droplets in the uterine glandular cells. A few lipid droplets are scattered in the stroma. Sudan Black B method. X 500.

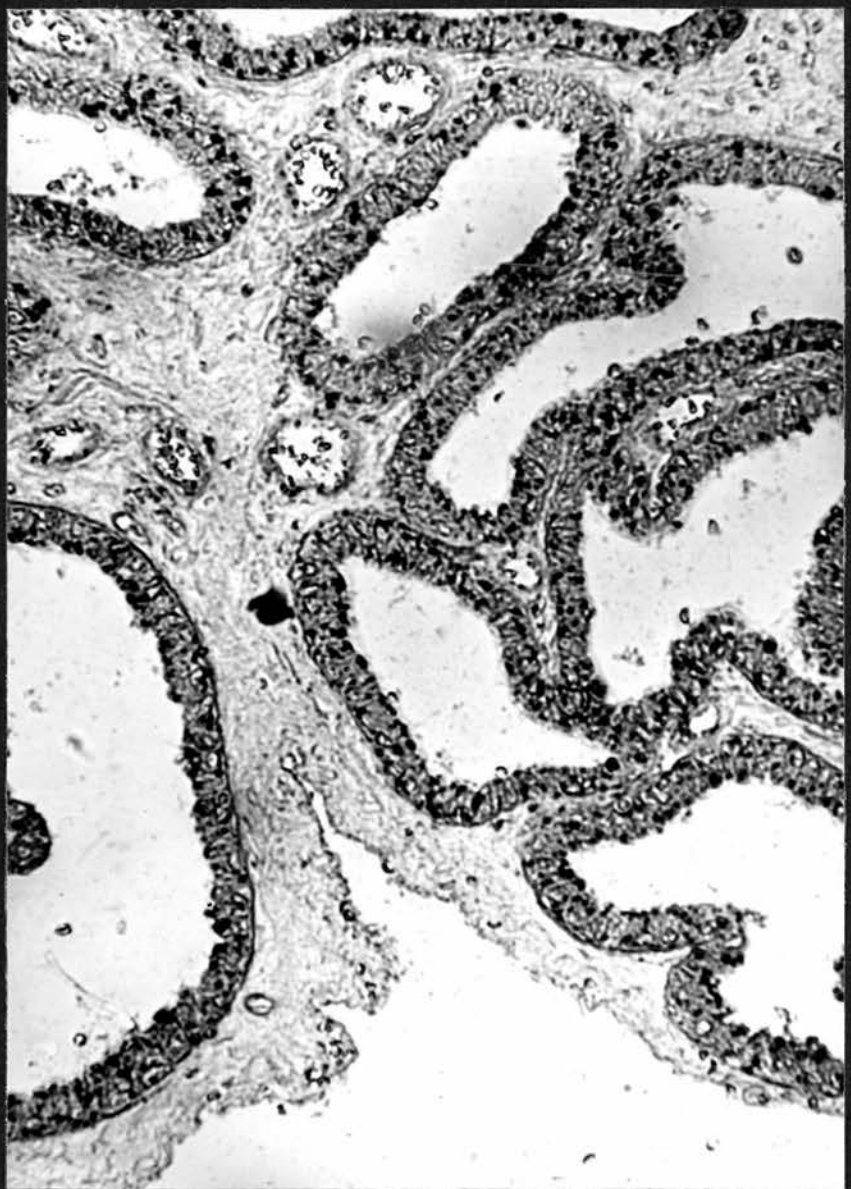


FIG. 62

Placenta - cotyledonary area (foetus 310 mm. C.R.L.).
Controlled chromation section.
Lipid droplets in the lining of a maternal septum and the chorionic epithelium of the villi.
Note the basal distribution of lipids in chorionic epithelium. Note also that the lipids are more in the maternal side than in the foetal side.

Sudan Black B method. X 500.

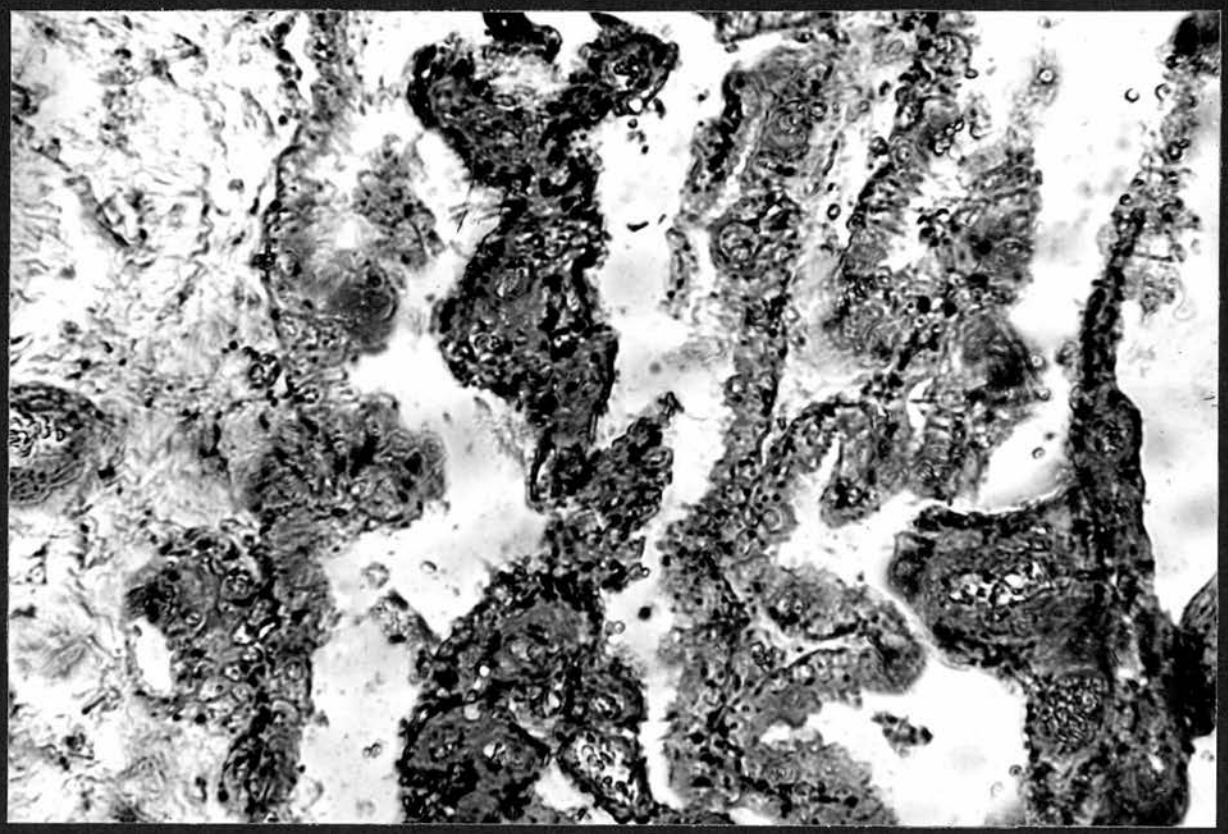


FIG. 63

Placenta - base of a villus (embryo 60 mm. C.R.L.).
Controlled chromation section.
Lipid droplets at the basal part of the chorionic columnar cells.

Sudan Black B method. X 500.

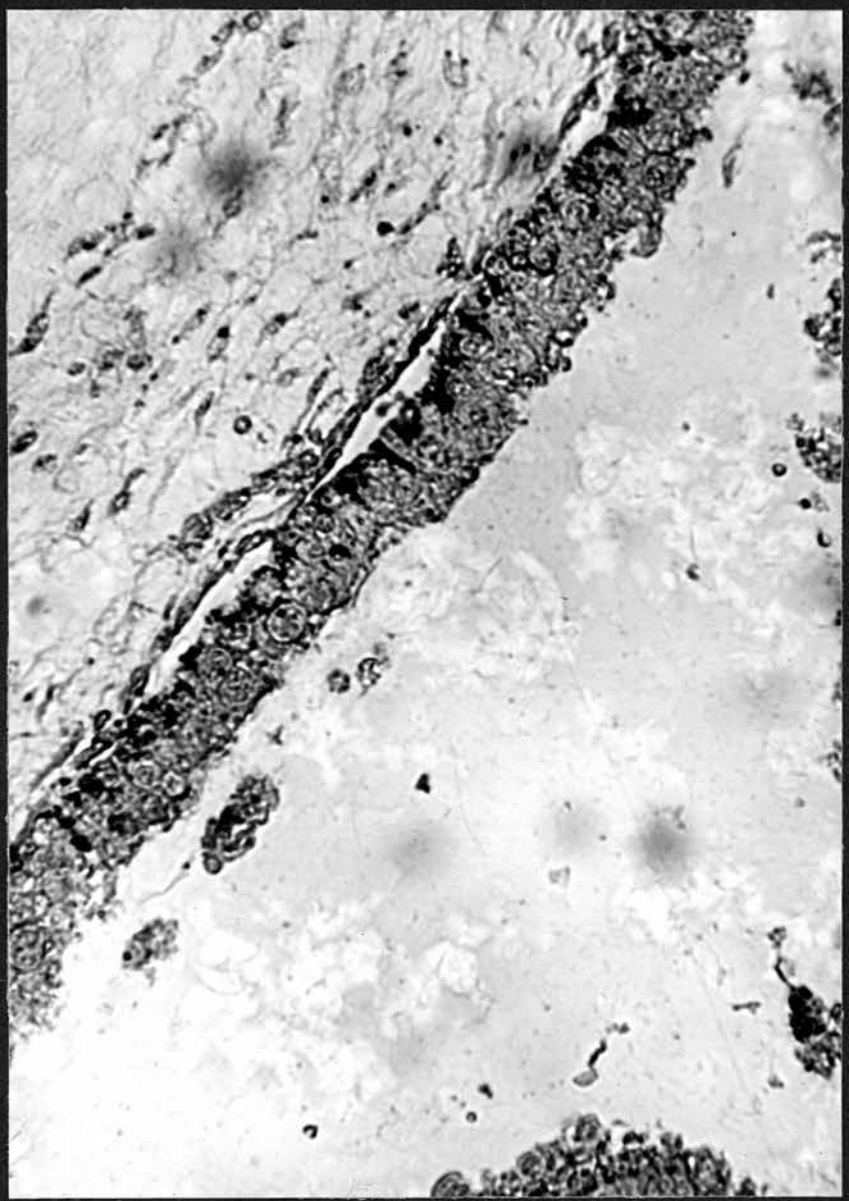


FIG. 64

Gravid horn - active uterine glands (120 days pregnancy approx.).

Ribonucleic acid in the glandular epithelial cells. Note the weak staining of the surface uterine epithelium.

Methyl Green Pyronin Y method. X 500.

FIG. 65

Placenta - tip of a villus and maternal crypt (embryo 60 mm. C.R.L.).

Ribonucleic acid in the chorionic epithelium and especially in the binucleate cells. The lining of the maternal crypts also shows a weak staining for ribonucleic acid.

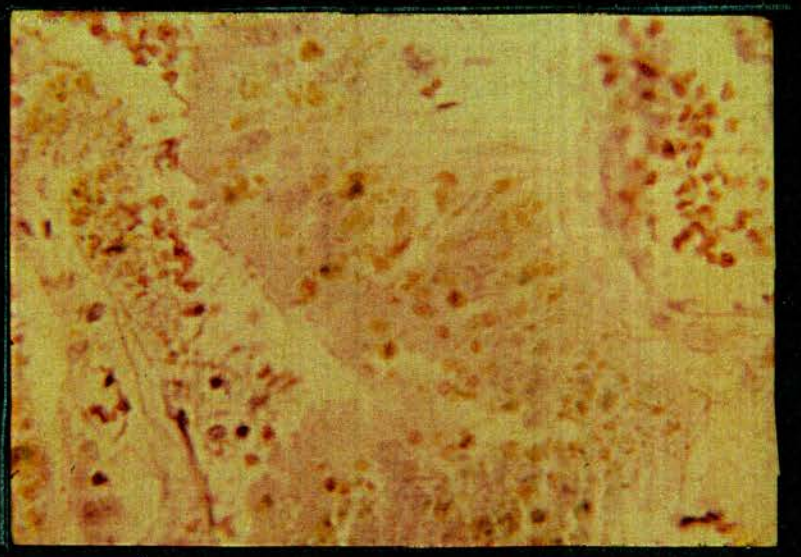
Methyl Green Pyronin Y method. X 500.

FIG. 66

Placenta - base of a villus (120 days pregnancy approx.). Freeze dried section.

Inorganic iron (Prussian blue reaction) and pigment granules in the chorionic columnar cells.

Perl's method. X 1000.



Figures 67 - 79 include electron micrographs of tubal epithelium and junctional zone of a fully developed placentome from a pregnant ewe (foetus 270 mm. C.R.L.).

FIG. 67

Electron micrograph of tubal epithelium of ampulla. Non-ciliated cells showing cytoplasmic projections which contain electron dense granules, vesicles and mitochondria.

X 32,000.

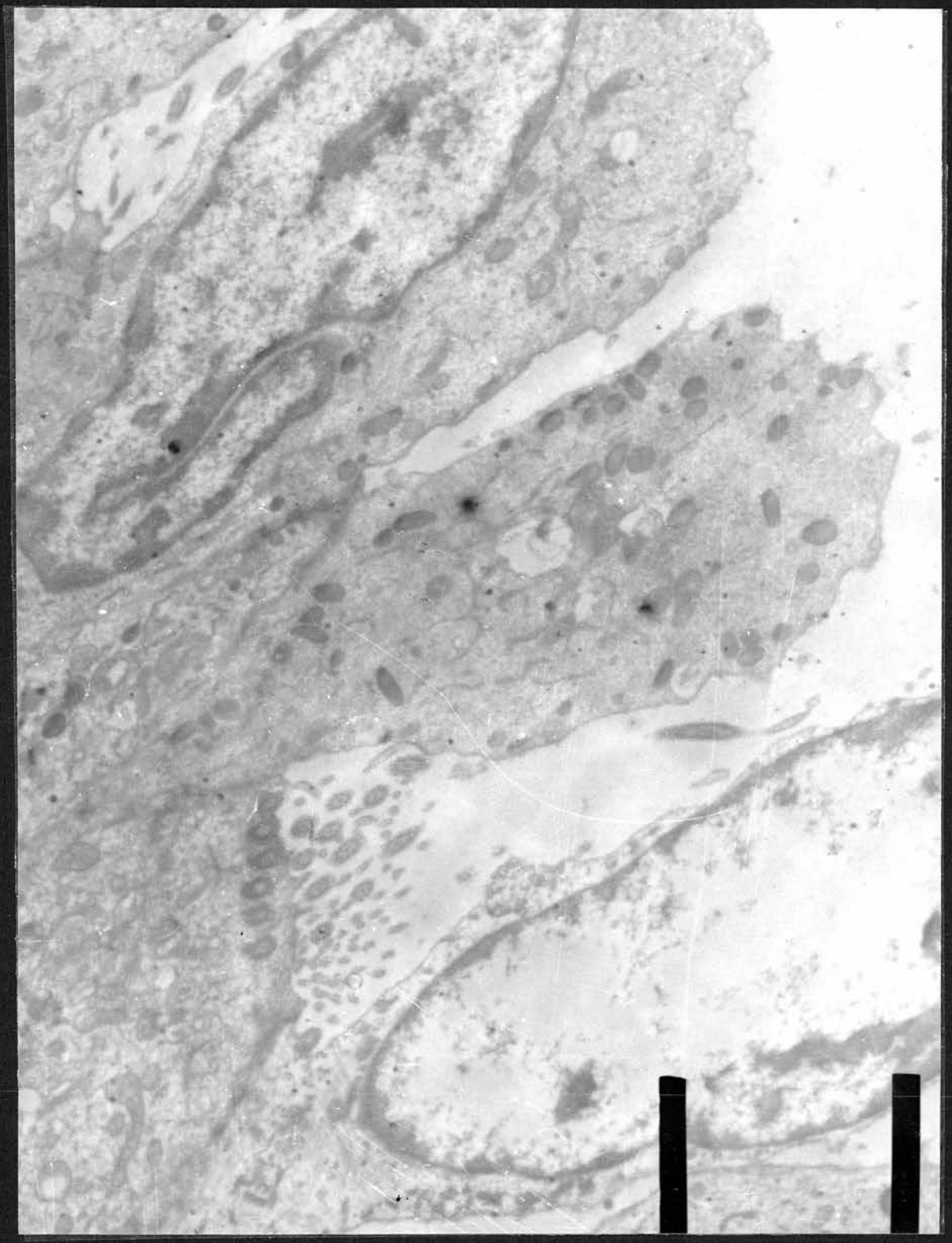


FIG. 68

Electron micrograph of tubal epithelium of ampulla. Groups of ciliated and non-ciliated cells. The epithelium appears pseudostratified. Note the nucleated cytoplasmic projection.

X 8,500.

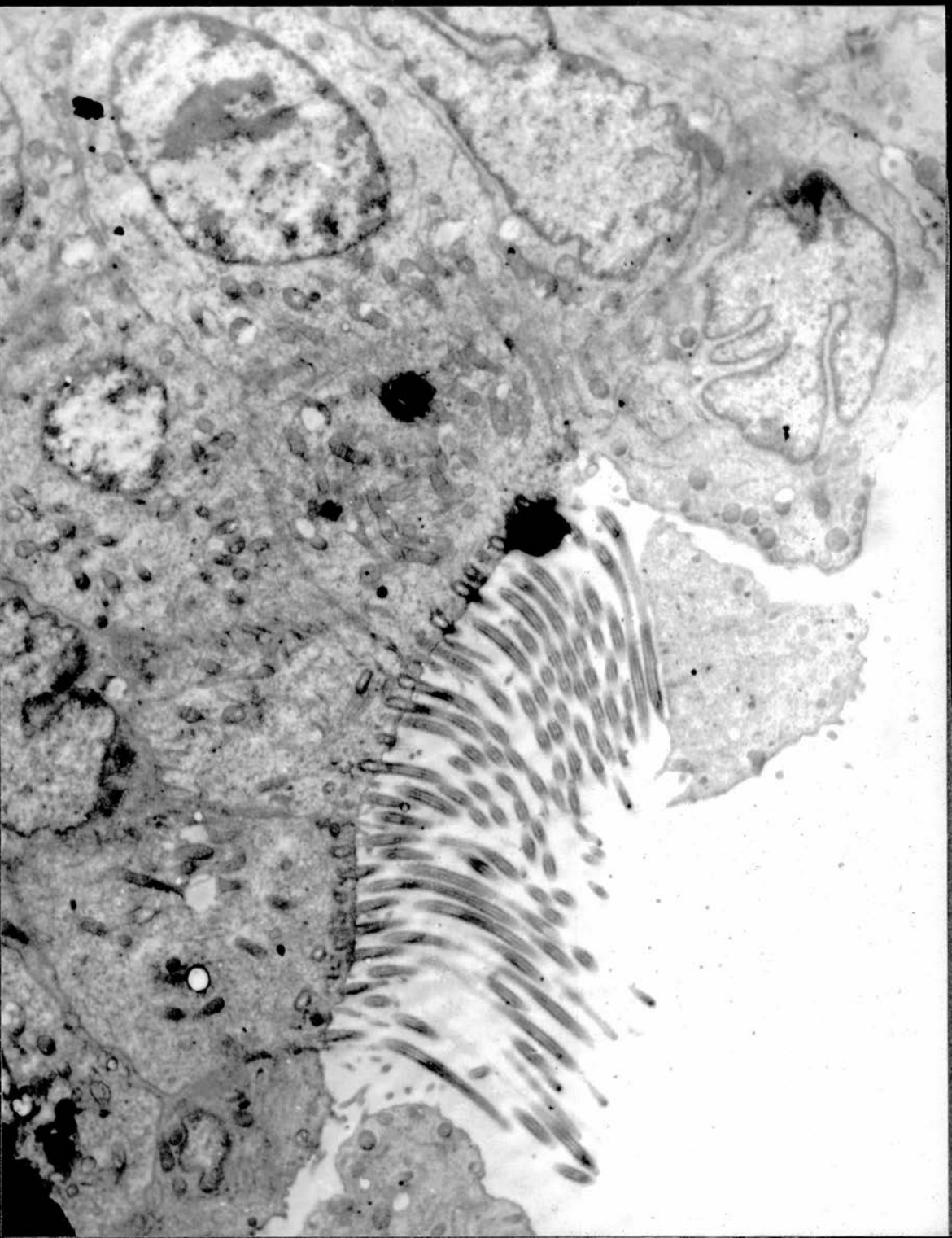


FIG. 69

Electron micrograph of tubal epithelium of ampulla. Microvilli and cilia of a ciliated cell. A cytoplasmic projection is seen free in the lumen, containing dense granules and endoplasmic reticulum.

X 20,000

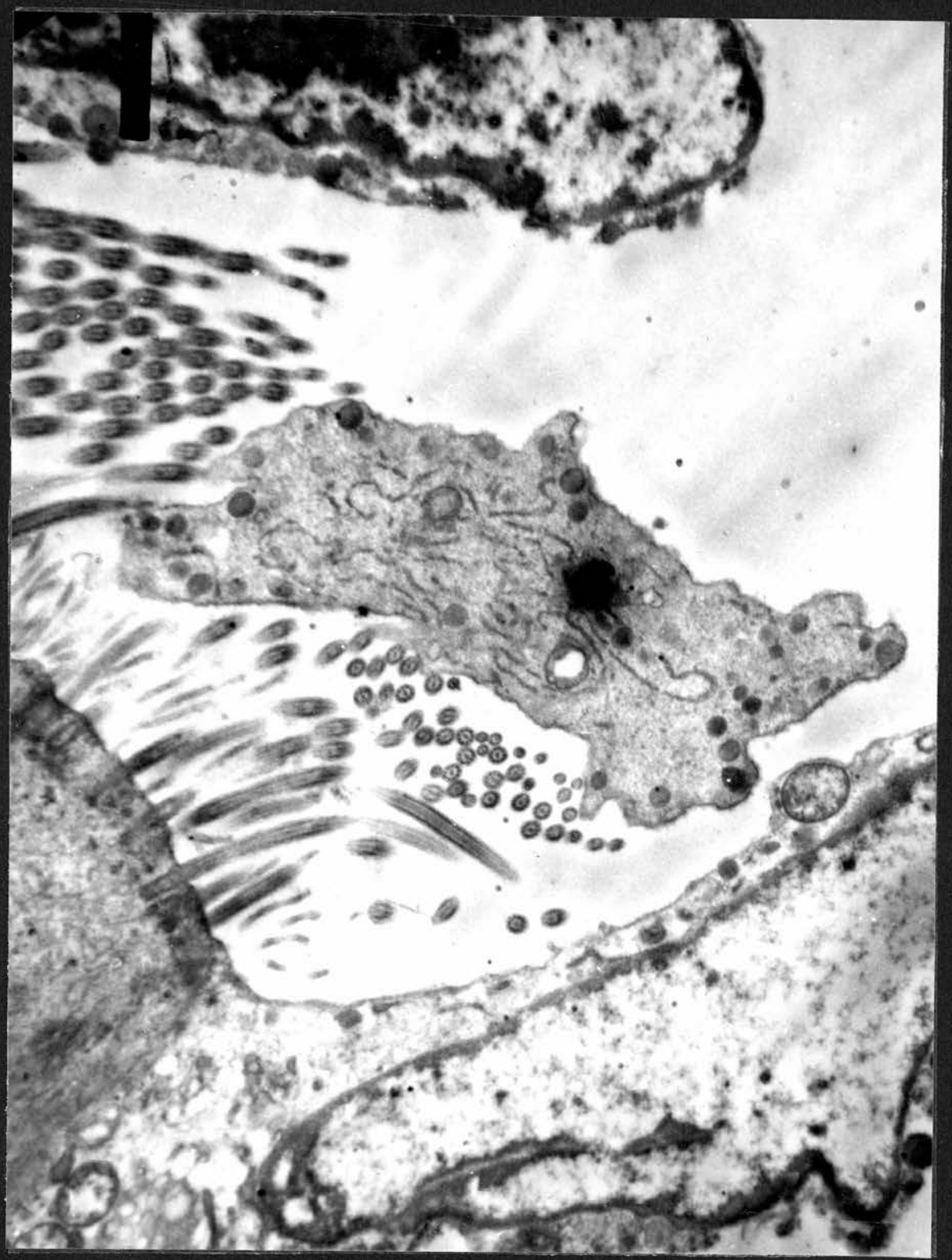


FIG. 70

Electron micrograph of tubal epithelium of ampulla. Non-ciliated tubal cells showing irregular protrusions like microvilli from their free surface. X 16,000.

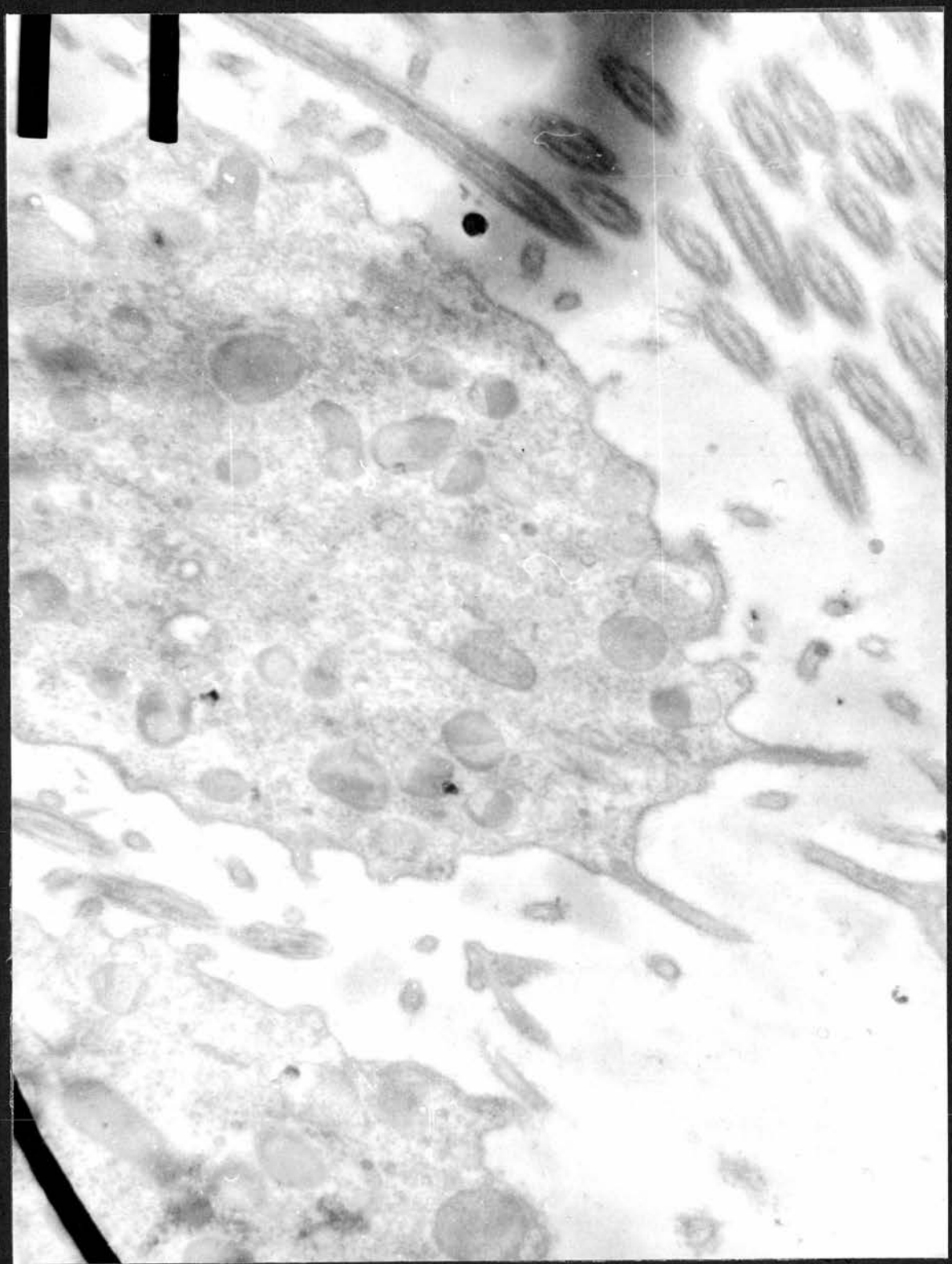


FIG. 71

Electron micrograph of tubal epithelium of ampulla.
An apical part of a ciliated cell showing cilia in longitudinal and transverse sections.
Note the central and peripheral dark bands in the cilia.
Note also the microvilli between the cilia.

X 24,000.

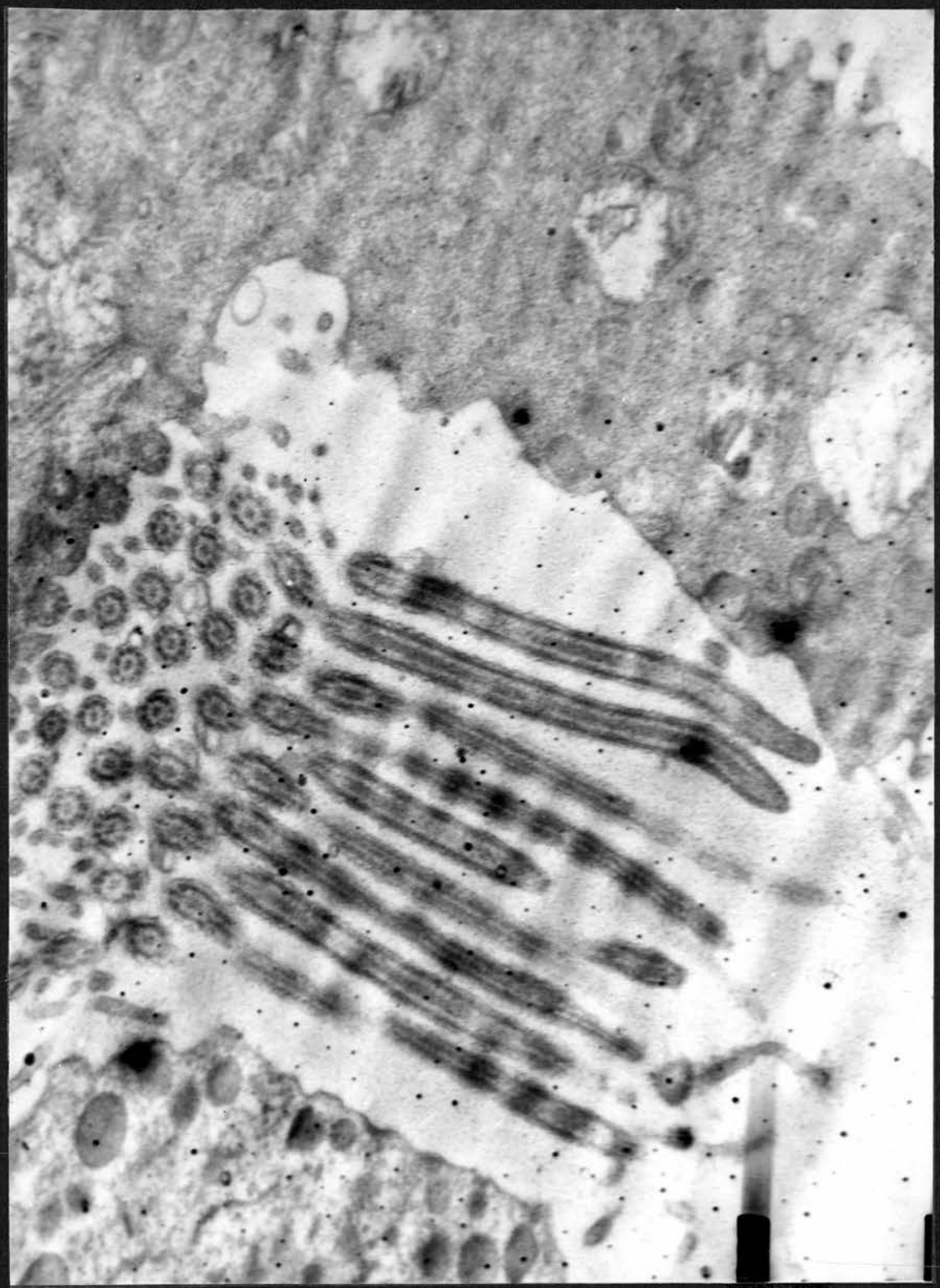


FIG. 72

Electron micrograph of tubal epithelium of ampulla. An apical part of a ciliated cell showing basal bodies of the cilia. Note the microvilli between the cilia are continuous with the plasma membrane.

X 15,000.

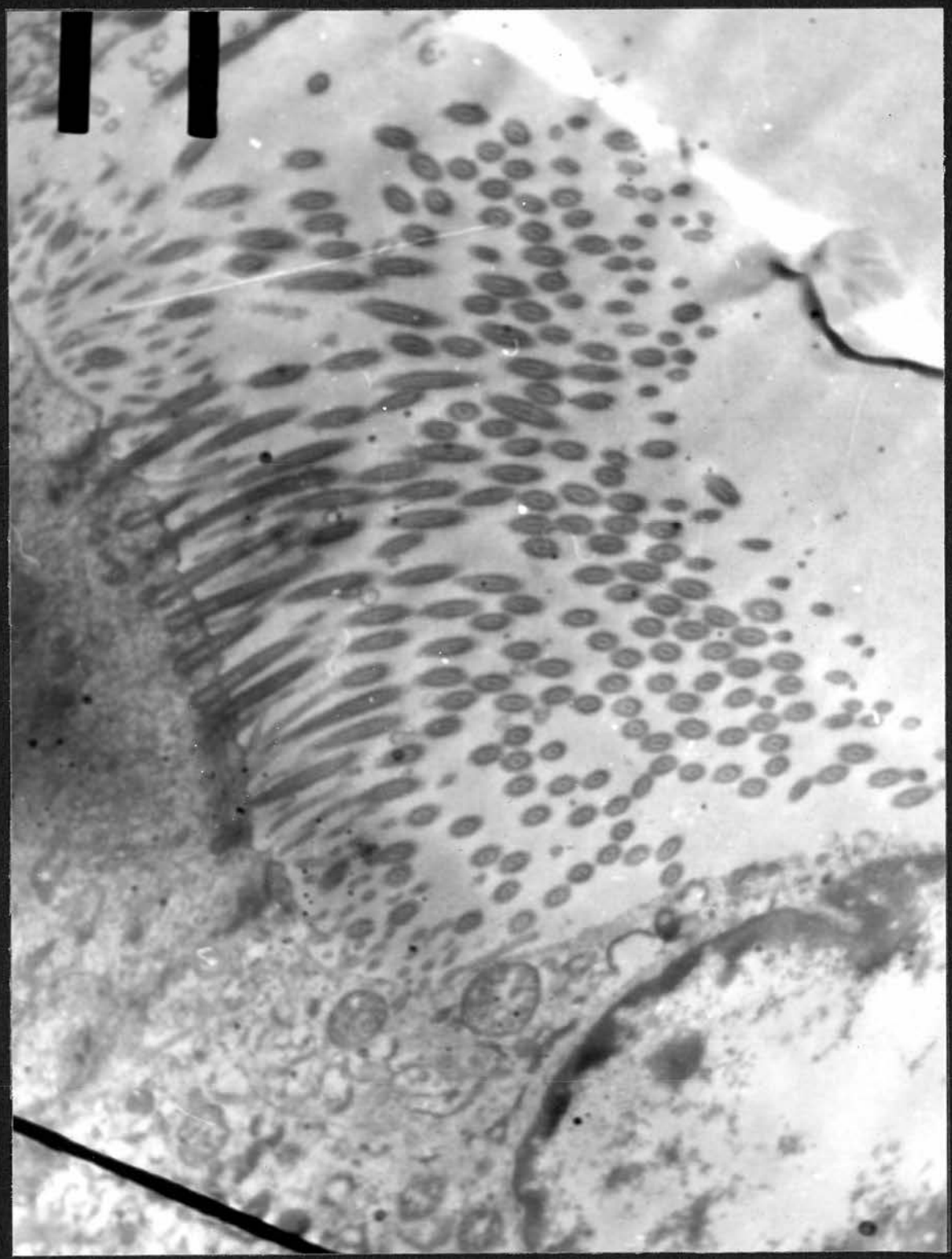


FIG. 73

Electron micrograph of a junctional zone of a fully developed placentome.

Interdigitations of apical microvilli of foetal and maternal lining cells. Note the large vesicles at the apical part of the chorionic epithelial cells.

X 13,500.

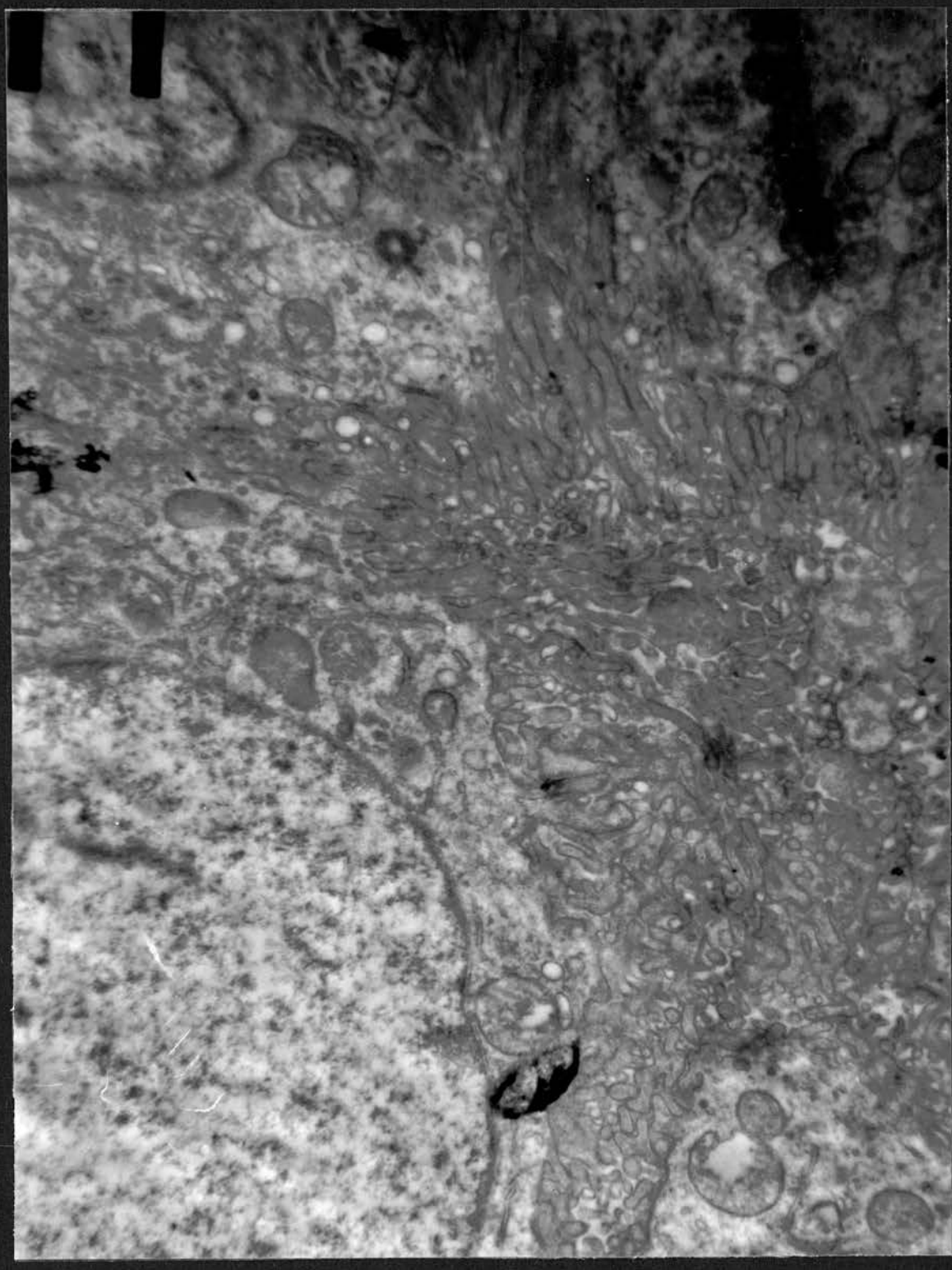


FIG. 74

Electron micrograph of a junctional zone of a fully developed placentome.

Electron dense granules and debris between the foetal and maternal lining cells. Note the absence of apical microvilli. Note also infoldings of the basal plasma membrane near a maternal capillary.

(N.B. The basal plasma membrane belongs to a binucleate cell in the next figure.)

X 4,000.

FIG. 75

Electron micrograph of a junctional zone of a fully developed placentome.

Binucleate cell in the lining of a maternal crypt showing short microvilli which interdigitate with the foetal lining cells.

X 4,000.

FIG. 76

Electron micrograph of a junctional zone of a fully developed placentome.

Binucleate cell in the lining of a foetal villus. Note that it possesses no microvilli and is partly covered by a cytoplasmic process of a neighbouring cell.

X 7,000.

FIG. 77

Higher power electron micrograph of Figure 76. Note the electron dense granules, vesicles and endoplasmic reticulum in the binucleate cell.

X 16,000.

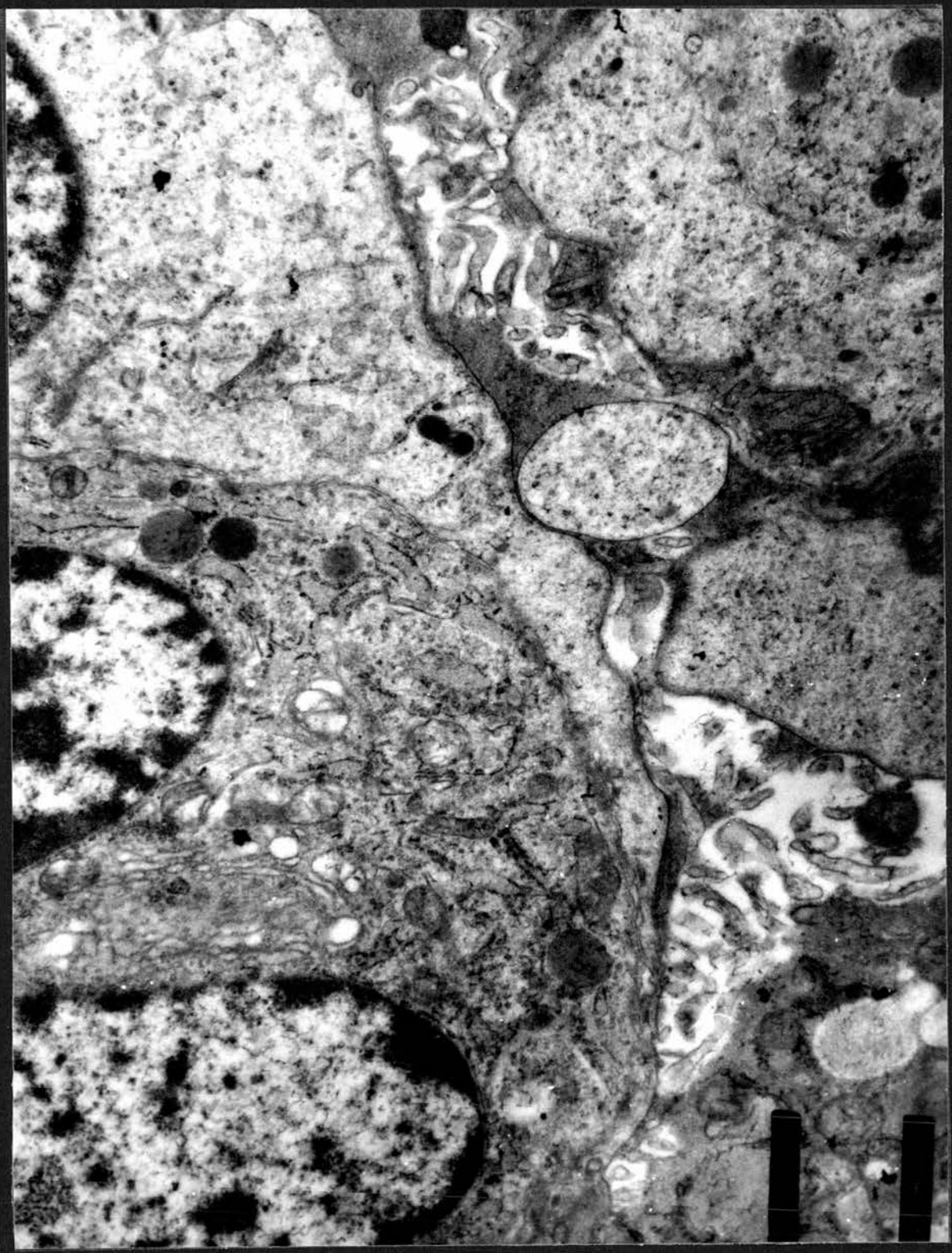


FIG. 78

High power electron micrograph of a maternal capillary and a part of a crypt lining cell.

Note the intervening space between the endothelium of the capillary and the neighbouring cell. Note also the granular appearance of the endoplasmic reticulum in the crypt lining cell.

X 25,000.

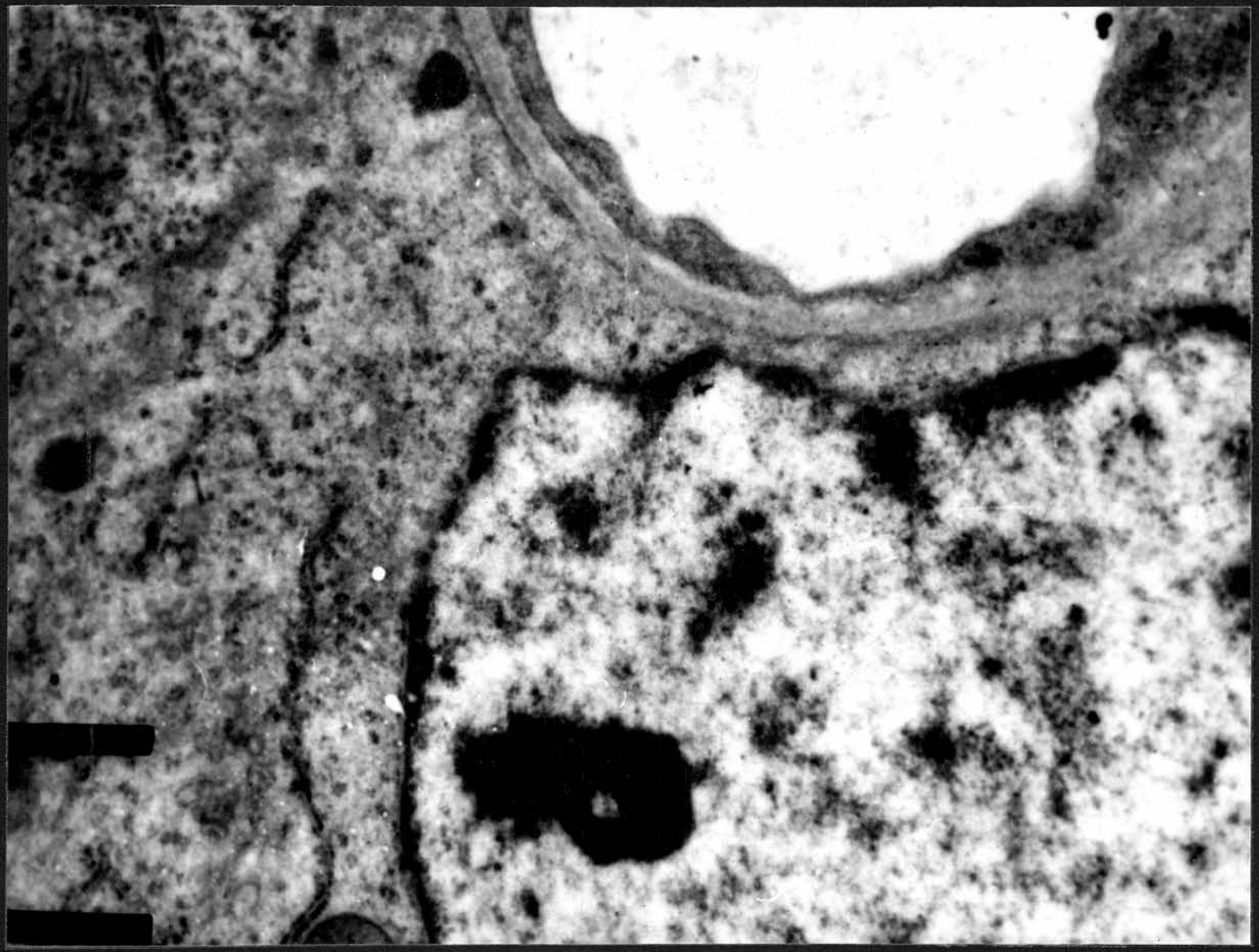


FIG. 79

Electron micrograph of a junctional zone of a fully developed placentome.

A syncytial lining of a maternal septum. Note the apical microvilli of the foetal lining partly interlocking with the syncytial lining. Note also the electron density of the nuclei and compare with those of the binucleate cell in Fig. 77.

X 7,000.